

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
SAU802654	SHA102148	56%	10.7%	89.4%
SAU802654	SHA103225	61%	24.3%	97.0%
SAU802654	SPN301653	43%	94.5%	7.0%
SAU802654	SPY200509	16%	52.0%	89.0%
SAU802654	TPA100484	21%	27.1%	39.4%
SAU802654	YPS002407	18%	61.5%	83.5%
SAU802655	BFR105074	22%	73.9%	84.7%
SAU802655	BCE107142	38%	90%	96.2%
SAU802655	BFU100048	43%	89.1%	98.0%
SAU802655	EBC101089	38%	72.2%	100%
SAU802655	EFM102193	42%	46.5%	99.0%
SAU802655	KPN303589	43%	82.2%	87.7%
SAU802655	PPU103404	39%	90%	98.5%
SAU802655	PSY103942	41%	82.6%	87.0%
SAU802655	SAU802655	100%	100%	100%
SAU802655	SEP201777	93%	99.1%	99.6%
SAU802658	SAU802658	100%	100%	100%
SAU802658	SEP201781	72%	99.2%	100%
SAU802658	SHA100132	66%	100%	100%
SAU802662	BFR102810	23%	65.5%	68.1%
SAU802662	CAC101079	35%	78.8%	79.1%
SAU802662	CAC101508	33%	99.6%	99.2%
SAU802662	CBO100561	36%	78.8%	78.2%
SAU802662	EFM202294	43%	82.0%	82.7%
SAU802662	SAU802662	100%	100%	100%
SAU802681	CAC101008	23%	48.4%	63.1%
SAU802681	CDF102990	31%	19.3%	28.0%
SAU802681	EFM102460	38%	61.8%	99.2%
SAU802681	SAU802681	100%	100%	100%
SAU802681	SHA103018	64%	99.7%	100%
SAU802681	SPN300982	23%	20.9%	18.3%
SAU802686	CDF102022	36%	17.7%	18.1%
SAU802686	ECO204773	48%	97.9%	91.7%
SAU802686	HPY100700	49%	97.9%	91.7%
SAU802686	LMO101556	57%	97.2%	97.9%
SAU802686	SAU802686	100%	100%	100%
SAU802686	SMU100471	52%	97.9%	98.2%
SAU802686	SPN400428	53%	97.9%	94.8%
SAU802689	ABA104423	38%	71.7%	72.6%
SAU802689	BAN106956	65%	91.8%	93.6%
SAU802689	BAN108132	66%	94.7%	94.4%
SAU802689	BPT101446	35%	70.8%	93.4%
SAU802689	BCE111516	32%	56.6%	91.7%
SAU802689	BFU112966	31%	70.8%	73.7%
SAU802689	BMA107217	31%	71.7%	78.6%
SAU802689	CPN200011	35%	91.2%	89.2%
SAU802689	CTR200904	37%	92.8%	89.6%
SAU802689	CDP100811	35%	83.6%	88.8%
SAU802689	EBC101390	30%	88.1%	80.5%
SAU802689	EFA200985	75%	96.9%	97.5%
SAU802689	EFM201522	70%	97.2%	98.7%
SAU802689	ECO101028	32%	88.1%	80.3%
SAU802689	KPN300416	27%	34.6%	71.3%
SAU802689	KPN302517	32%	89.0%	80.2%

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Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
SAU802689	LPN102475	44%	39.0%	97.7%
SAU802689	LMO100379	69%	96.5%	95.6%
SAU802689	PAE200857	37%	71.7%	72.8%
SAU802689	PPU109900	39%	71.7%	73.2%
SAU802689	PSY103191	39%	71.7%	71.6%
SAU802689	SPA101128	33%	65.7%	85.8%
SAU802689	STY102479	30%	88.1%	80.3%
SAU802689	STM102282	30%	88.1%	80.3%
SAU802689	SAU802689	100%	100%	100%
SAU802689	SEP202172	77%	99.7%	99.1%
SAU802689	SHA102497	82%	100%	99.4%
SAU802689	SPN400084	63%	95.9%	94.5%
SAU802689	SPY200686	64%	95.9%	94.5%
SAU802689	VCH101240	35%	72.3%	69.0%
SAU802689	YPS003127	31%	87.4%	78.6%
SAU802701	ABA102929	41%	87.3%	96.5%
SAU802701	BAN109528	29%	25.8%	95.5%
SAU802701	BAN100158	36%	38.5%	79.9%
SAU802701	BAN112678	26%	80.2%	78.6%
SAU802701	BAN110028	50%	40.9%	99.0%
SAU802701	BAN108206	48%	87.3%	22.2%
SAU802701	BAN101564	51%	69.8%	97.8%
SAU802701	BAN109552	43%	87.7%	95.7%
SAU802701	BAN111328	45%	97.2%	98.8%
SAU802701	BAN110379	47%	96.8%	96.8%
SAU802701	BAN108174	48%	97.2%	97.6%
SAU802701	BAN101135	49%	98.8%	100%
SAU802701	BAN112693	49%	100%	98.1%
SAU802701	BAN108774	51%	99.6%	97.7%
SAU802701	BCE101081	43%	86.5%	94.4%
SAU802701	BFU106323	40%	86.5%	72.9%
SAU802701	CAC100479	49%	100%	99.6%
SAU802701	CDF101638	49%	98.0%	99.2%
SAU802701	CDP101466	47%	81.0%	91.0%
SAU802701	EFA200808	49%	97.2%	96.1%
SAU802701	ECO100486	38%	87.7%	97.4%
SAU802701	KPN302594	39%	88.1%	95.7%
SAU802701	LMO102938	50%	99.6%	98.8%
SAU802701	MBV100633	36%	91.3%	93.1%
SAU802701	MTU200975	36%	91.3%	93.1%
SAU802701	MGE100067	45%	80.6%	43.1%
SAU802701	MPN100074	44%	80.6%	43.2%
SAU802701	PMU100448	41%	90.1%	96.9%
SAU802701	SPA101078	36%	80.2%	98.1%
SAU802701	SAU802701	100%	100%	100%
SAU802701	SEP201315	71%	100%	100%
SAU802701	SHA102331	72%	100%	100%
SAU802701	SPY201472	44%	94.4%	96.3%
SAU802701	VCH103502	39%	88.5%	98.7%
SAU802710	ABA100898	30%	79.1%	85.7%
SAU802710	BAN112555	58%	97.5%	100%
SAU802710	BAN106936	64%	98.7%	99.2%
SAU802710	BFR100717	35%	59.4%	66.5%
SAU802710	BPT103217	29%	67.4%	71.3%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
SAU802710	BBU100176	26%	72.0%	81.2%
SAU802710	BCE112346	30%	87.4%	89.9%
SAU802710	BFU105165	30%	84.5%	92.5%
SAU802710	BMA104306	30%	73.2%	76.3%
SAU802710	CJU100925	26%	68.2%	82.4%
SAU802710	CAC102710	43%	95.8%	96.2%
SAU802710	CBO101450	39%	93.3%	93.7%
SAU802710	CDF100026	49%	36.8%	88.1%
SAU802710	CDF104563	50%	89.1%	100%
SAU802710	CDP101653	40%	52.3%	59.2%
SAU802710	EBC100304	32%	78.2%	96.2%
SAU802710	EFA200737	62%	98.7%	99.6%
SAU802710	EFM200318	61%	98.7%	97.5%
SAU802710	ECO103661	34%	85.8%	93.7%
SAU802710	HIN100467	32%	87.4%	97.5%
SAU802710	HPY101046	33%	64.9%	83.7%
SAU802710	KPN301111	35%	87.4%	97.5%
SAU802710	LPN100764	33%	62.8%	69.2%
SAU802710	LMO101197	59%	98.7%	99.6%
SAU802710	MCA101113	31%	72.0%	76.9%
SAU802710	MAV100553	33%	49.8%	47.6%
SAU802710	MBV105240	32%	46.0%	48.7%
SAU802710	MLP101601	30%	69.5%	65.7%
SAU802710	MTU203864	31%	46.0%	48.7%
SAU802710	MGE100391	30%	62.8%	76.6%
SAU802710	MPN100284	28%	63.2%	77.5%
SAU802710	NGO100788	29%	84.5%	92.3%
SAU802710	NME200072	28%	84.9%	92.8%
SAU802710	PMU101486	34%	85.8%	92.4%
SAU802710	PRT105128	35%	74.1%	82.7%
SAU802710	PAE205559	28%	87.4%	93.0%
SAU802710	PPU102171	28%	89.5%	94.9%
SAU802710	PSY102503	29%	73.2%	79.0%
SAU802710	SPA103410	31%	89.1%	97.6%
SAU802710	STY103852	31%	89.1%	97.6%
SAU802710	SAU802710	100%	100%	100%
SAU802710	SEP201294	81%	99.6%	99.6%
SAU802710	SHA101597	82%	98.7%	98.3%
SAU802710	SMU100460	55%	98.7%	100%
SAU802710	SPN401163	53%	98.7%	96.7%
SAU802710	SPY200240	55%	98.7%	100%
SAU802710	TPA100936	27%	66.1%	70.7%
SAU802710	UUR100038	33%	88.7%	99.1%
SAU802710	VCH102735	32%	87.0%	94.3%
SAU802710	YPS000008	34%	83.7%	92.2%
SAU802711	ABA101587	49%	98.9%	98.6%
SAU802711	BAN106565	54%	99.0%	99.7%
SAU802711	BAN109722	76%	99.4%	98.7%
SAU802711	BFR10096	45%	97.6%	98.9%
SAU802711	BPT100036	48%	98.4%	97.8%
SAU802711	BBU100177	48%	98.6%	97.4%
SAU802711	BCE103020	47%	99.0%	96.2%
SAU802711	BFU109113	48%	98.4%	96.2%
SAU802711	BMA106271	48%	99.0%	96.0%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
SAU802711	CJU101113	46%	98.4%	99.4%
SAU802711	CPN200128	48%	98.1%	98.2%
SAU802711	CTR200774	47%	98.2%	98.5%
SAU802711	CAC101884	54%	98.1%	97.9%
SAU802711	CBO102626	55%	98.2%	97.0%
SAU802711	CDF103119	56%	98.2%	97.3%
SAU802711	EBC101931	52%	98.1%	99.0%
SAU802711	EFA200717	66%	99.4%	98.7%
SAU802711	EFM202552	66%	99.4%	98.4%
SAU802711	ECO103662	51%	98.9%	98.7%
SAU802711	HIN100562	50%	99.2%	99.0%
SAU802711	HPY100209	46%	99.0%	99.7%
SAU802711	KPN300377	54%	29.6%	93.4%
SAU802711	KPN301110	50%	95.4%	100%
SAU802711	LPN101818	49%	98.9%	99.0%
SAU802711	LMO102246	71%	99.2%	98.6%
SAU802711	MCA101156	47%	98.9%	97.6%
SAU802711	MGE100390	44%	97.6%	98.9%
SAU802711	MPN100285	41%	97.9%	99.2%
SAU802711	NGO100779	47%	98.2%	97.8%
SAU802711	NME200069	47%	98.2%	98.2%
SAU802711	PMU101485	51%	99.2%	99.0%
SAU802711	PRT103989	51%	99.2%	98.9%
SAU802711	PAE205560	50%	98.1%	97.3%
SAU802711	PPU103677	50%	97.8%	98.2%
SAU802711	PSY103430	49%	57.1%	97.0%
SAU802711	SPA103411	50%	98.9%	99.7%
SAU802711	STY103850	51%	98.9%	98.7%
SAU802711	SAU802711	100%	100%	100%
SAU802711	SEP201293	93%	99.7%	99.7%
SAU802711	SHA101596	94%	100%	100%
SAU802711	SMU101356	63%	98.2%	97.8%
SAU802711	SPN400124	63%	99.5%	98.0%
SAU802711	SPY201670	63%	99.2%	98.7%
SAU802711	TPA100043	42%	98.2%	99.0%
SAU802711	UUR100037	46%	98.9%	99.7%
SAU802711	VCH102736	49%	98.4%	97.8%
SAU802711	YPS000011	50%	99.0%	98.9%
SAU802713	ABA100989	26%	83.5%	73.7%
SAU802713	BAN108182	50%	89.6%	90.4%
SAU802713	BAN113266	51%	91.3%	89.1%
SAU802713	BFR101294	30%	64.3%	55.8%
SAU802713	BPT105773	40%	35.7%	34.1%
SAU802713	BBU100440	23%	82.6%	82.4%
SAU802713	BMA108699	29%	69.6%	51.0%
SAU802713	CJU100890	23%	95.7%	99.1%
SAU802713	CPN200910	34%	72.2%	59.0%
SAU802713	CTR200162	26%	71.3%	68.9%
SAU802713	CAC102336	38%	91.3%	89.9%
SAU802713	CBO103162	37%	94.8%	99.1%
SAU802713	CDF103808	40%	86.1%	87.7%
SAU802713	CDP101660	29%	54.8%	53.8%
SAU802713	EBC100049	28%	70.4%	89.0%
SAU802713	EFA200678	44%	96.5%	94.9%



Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
SAU802713	EFM102258	42%	60.9%	97.3%
SAU802713	ECO103625	26%	83.5%	80.7%
SAU802713	HIN100980	28%	69.6%	67.2%
SAU802713	HPY101427	27%	94.8%	80.7%
SAU802713	KPN302213	32%	55.7%	54.6%
SAU802713	LPN100279	32%	96.5%	96.5%
SAU802713	LMO102928	52%	98.3%	95.8%
SAU802713	MCA103188	32%	73.0%	62.0%
SAU802713	MBV103340	24%	88.7%	81.6%
SAU802713	MLP101604	21%	89.6%	92.5%
SAU802713	MTU203868	24%	88.7%	81.6%
SAU802713	MGE100474	28%	86.1%	89.8%
SAU802713	NGO101432	28%	88.7%	85.1%
SAU802713	NME200510	28%	88.7%	85.1%
SAU802713	PMU101163	29%	64.3%	56.9%
SAU802713	PRT102997	33%	55.7%	54.6%
SAU802713	PAE205564	31%	70.4%	60.7%
SAU802713	PPU102196	38%	42.6%	30.3%
SAU802713	PSY107592	33%	64.3%	43.6%
SAU802713	STY104781	27%	83.5%	80.7%
SAU802713	STM104793	27%	83.5%	80.7%
SAU802713	SAU802713	100%	100%	100%
SAU802713	SEP201290	83%	100%	100%
SAU802713	SHA100141	83%	100%	100%
SAU802713	SMU100665	44%	97.4%	93.3%
SAU802713	SPN401851	45%	97.4%	91.1%
SAU802713	SPY200178	46%	97.4%	93.3%
SAU802713	UUR100610	31%	93.0%	92.9%
SAU802713	VCH100006	33%	76.5%	74.6%
SAU802713	YPS003466	33%	56.5%	55.5%
SAU802714	ABA100986	62%	95.6%	97.7%
SAU802714	BAN102648	88%	97.8%	100%
SAU802714	BAN102568	88%	97.8%	100%
SAU802714	BFR106080	62%	95.6%	91.5%
SAU802714	BPT100632	53%	95.6%	97.7%
SAU802714	BBU100439	55%	95.6%	84.3%
SAU802714	BCE105407	55%	95.6%	97.7%
SAU802714	BMA109034	55%	95.6%	97.7%
SAU802714	CJU100891	60%	95.6%	97.7%
SAU802714	CPN200909	64%	93.3%	93.3%
SAU802714	CTR200163	61%	93.3%	93.3%
SAU802714	CAC101976	70%	91.1%	93.2%
SAU802714	CBO101345	63%	91.1%	70.7%
SAU802714	CDF104319	70%	97.8%	97.8%
SAU802714	CDP101663	58%	95.6%	86%
SAU802714	EBC100048	62%	95.6%	93.5%
SAU802714	EFA202663	86%	97.8%	100%
SAU802714	ECO103624	62%	95.6%	93.5%
SAU802714	HIN100979	70%	97.8%	100%
SAU802714	HPY101426	60%	95.6%	97.7%
SAU802714	KPN204278	62%	95.6%	93.5%
SAU802714	LPN103600	61%	97.8%	100%
SAU802714	LMO100377	84%	97.8%	100%
SAU802714	MCA103596	60%	95.6%	97.7%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
SAU802714	MBV102731	59%	93.3%	91.3%
SAU802714	MLP101605	58%	95.6%	91.5%
SAU802714	MTU203869	60%	95.6%	91.5%
SAU802714	MGE100475	58%	95.6%	89.6%
SAU802714	MPN100160	58%	95.6%	89.6%
SAU802714	NGO103743	55%	95.6%	97.7%
SAU802714	NME200511	55%	95.6%	97.7%
SAU802714	PMU101162	70%	97.8%	100%
SAU802714	PRT102996	60%	95.6%	91.5%
SAU802714	PAE205565	65%	95.6%	97.7%
SAU802714	PPU102199	62%	95.6%	97.7%
SAU802714	SPA101308	62%	95.6%	93.5%
SAU802714	STY104683	62%	95.6%	93.5%
SAU802714	STM104752	62%	95.6%	93.5%
SAU802714	SAU802714	100%	100%	100%
SAU802714	SEP201288	100%	100%	100%
SAU802714	SHA100140	100%	100%	100%
SAU802714	SMU102293	72%	97.8%	100%
SAU802714	SPN401805	72%	97.8%	100%
SAU802714	SPY200181	75%	97.8%	100%
SAU802714	TPA100941	55%	95.6%	84.3%
SAU802714	UUR100611	72%	95.6%	89.6%
SAU802714	VCH100007	63%	97.8%	97.8%
SAU802714	YPS003462	60%	95.6%	93.5%
STM100137	BPT103857	29%	85.4%	59.3%
STM100137	BFU113310	31%	82.2%	80.5%
STM100137	BMA106841	30%	83.6%	48.9%
STM100137	PAE111701	33%	15.5%	64.5%
STM100137	PPU102760	32%	23.2%	28.5%
STM100137	SPA106841	87%	7.0%	70.9%
STM100137	STM100137	100%	100%	100%
STM100137	SAU501115	27%	19.6%	25.6%
STM100227	BCE105792	22%	31.0%	52.5%
STM100227	BCE101253	29%	25.5%	38.4%
STM100227	BFU102165	29%	15.8%	32.0%
STM100227	KPN109637	48%	15.6%	92.1%
STM100227	STM100227	100%	100%	100%
STM100227	SAU502668	32%	7.2%	20.1%
STM100229	BCE113297	41%	41.4%	100%
STM100229	BMA101920	35%	95.7%	91.3%
STM100229	CDP102420	32%	12.3%	94.7%
STM100229	KPN112130	68%	29.7%	89.1%
STM100229	MAV107220	28%	93.7%	93.8%
STM100229	PAE111649	34%	78.5%	92.8%
STM100229	SPA106013	100%	13.6%	74.4%
STM100229	STM100229	100%	100%	100%
STM100229	SMU101194	24%	17.2%	21.3%
STM100274	ABA101013	37%	89.8%	93.7%
STM100274	ABA100365	41%	89.8%	93.7%
STM100274	BPT100083	36%	96.5%	100%
STM100274	BCE104632	39%	81.9%	100%
STM100274	BFU102105	41%	92.7%	96.1%
STM100274	BMA108617	42%	90.5%	94.0%
STM100274	EBC100961	77%	99.7%	98.7%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
STM100274	ECO101491	84%	98.7%	96.9%
STM100274	KPN306347	56%	96.5%	100%
STM100274	LPN103390	29%	69.5%	95.5%
STM100274	MCA100493	34%	96.5%	100%
STM100274	MLP100143	35%	15.2%	8.4%
STM100274	PRT101607	55%	94.0%	100%
STM100274	PPU106304	37%	94.3%	98.0%
STM100274	PSY103291	29%	81.3%	90.3%
STM100274	SPA103363	96%	98.7%	96.9%
STM100274	STY104266	99%	100%	100%
STM100274	STM100274	100%	100%	100%
STM100541	MAV101179	24%	26.4%	27.1%
STM100541	STM100541	100%	100%	100%
STM100580	BPT103484	40%	96.2%	68.4%
STM100580	BCE114868	44%	86.6%	96.9%
STM100580	BMA108364	24%	49.4%	48.6%
STM100580	PPU111837	30%	91.7%	71.4%
STM100580	SPA107052	72%	11.8%	86.0%
STM100580	STM100580	100%	100%	100%
STM100637	BMA101649	23%	82.3%	55.3%
STM100637	EBC104360	25%	17.3%	15.9%
STM100637	ECO103620	25%	14.5%	13.2%
STM100637	KPN301149	25%	17.3%	30.6%
STM100637	PRT102211	27%	11.9%	10.9%
STM100637	STY103968	25%	17.3%	15.9%
STM100637	STM101609	40%	97.6%	95.8%
STM100637	STM100637	100%	100%	100%
STM100638	BCE104424	35%	13.2%	39.6%
STM100638	STM100638	100%	100%	100%
STM100693	STM100290	28%	79.6%	80.3%
STM100693	STM100693	100%	100%	100%
STM100723	ABA104878	49%	99.2%	99.0%
STM100723	BAN106141	34%	43.8%	99.7%
STM100723	BAN102570	46%	43.8%	96.9%
STM100723	BFR101415	47%	42.9%	93.2%
STM100723	BPT101834	29%	44.5%	42.5%
STM100723	BBU100588	35%	42.6%	91.5%
STM100723	BCE113410	44%	36.6%	95.4%
STM100723	BFU103363	44%	44.3%	93.9%
STM100723	BMA102976	32%	43.7%	41.4%
STM100723	CJU100638	49%	45.4%	64.9%
STM100723	CAC102832	45%	44.1%	97.0%
STM100723	CBO102063	55%	22.4%	90.4%
STM100723	CDF100616	23%	44.4%	97.6%
STM100723	CDF100432	24%	43.8%	96.0%
STM100723	CDP101294	43%	59.5%	91.4%
STM100723	EBC104962	91%	99.9%	100%
STM100723	EFA202248	44%	42.6%	93.6%
STM100723	EFM200028	38%	26.9%	93.7%
STM100723	ECO102255	96%	99.9%	100%
STM100723	HIN101176	68%	99.6%	100%
STM100723	HPY200837	50%	30.5%	6.7%
STM100723	KPN305976	95%	99.3%	100%
STM100723	LPN102073	27%	26.3%	41.0%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
STM100723	LMO100950	47%	42.7%	93.8%
STM100723	MCA101508	64%	23.8%	1.4%
STM100723	MAV101929	36%	99.3%	98.6%
STM100723	MBV102834	36%	99.2%	98.8%
STM100723	MTU200407	36%	99.2%	98.8%
STM100723	MGE100305	35%	44.7%	97.5%
STM100723	MPN100413	36%	44.0%	97.2%
STM100723	NGO100804	70%	17.5%	8.2%
STM100723	NME200777	70%	17.5%	8.2%
STM100723	PMU100705	69%	99.6%	100%
STM100723	PRT100628	81%	99.3%	100%
STM100723	PAE200834	55%	98.9%	98.9%
STM100723	PPU103956	50%	98.6%	100%
STM100723	PSY102379	52%	97.5%	99.4%
STM100723	SPA102048	91%	70.3%	93.3%
STM100723	STY100983	99%	100%	100%
STM100723	STM100723	100%	100%	100%
STM100723	SAU800588	42%	43.0%	94.8%
STM100723	SEP200137	44%	43.0%	94.8%
STM100723	SHA101700	45%	43.0%	94.8%
STM100723	SMU100210	45%	42.6%	92.4%
STM100723	SPN401007	48%	43.1%	95.1%
STM100723	SPY200858	44%	43.3%	94.0%
STM100723	TPA100093	41%	43.3%	95.5%
STM100723	UUR100064	24%	35.4%	82.9%
STM100723	VCH101078	73%	100%	100%
STM100723	YPS000496	86%	99.6%	99.2%
STM100724	BAN104006	29%	73.1%	91.0%
STM100724	BAN106594	29%	97.8%	96.6%
STM100724	BFR103701	31%	95.7%	97.4%
STM100724	BPT103061	21%	19.2%	22.9%
STM100724	BBU100842	29%	95.7%	96.3%
STM100724	BMA107628	29%	77.9%	72.5%
STM100724	CTR100660	29%	20.8%	36.2%
STM100724	CBO103750	28%	98.4%	98.8%
STM100724	CDF101574	35%	76.5%	87.3%
STM100724	CDF101198	35%	98.0%	97.0%
STM100724	CDP101707	39%	11.5%	12.1%
STM100724	EBC103307	30%	96.0%	98.3%
STM100724	EFA202432	26%	80.6%	87.0%
STM100724	EFM200181	50%	5.1%	15.1%
STM100724	ECO102256	91%	100%	98.6%
STM100724	HIN100573	28%	97.2%	97.8%
STM100724	HPY100684	24%	35.2%	38.1%
STM100724	MCA100770	21%	72.7%	73.0%
STM100724	PMU101104	23%	34.0%	34.4%
STM100724	PRT100826	29%	96.4%	96.4%
STM100724	SPA102153	96%	97.8%	100%
STM100724	STY100984	100%	100%	100%
STM100724	STM100724	100%	100%	100%
STM100724	SAU801171	29%	97.6%	97.3%
STM100724	SEP202232	28%	97.4%	96.5%
STM100724	SHA102381	30%	97.6%	98.1%
STM100724	SMU100142	21%	76.7%	79.7%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
STM100724	SPN401956	28%	98.2%	98.2%
STM100724	SPY201191	27%	98.2%	99.4%
STM100724	VCH100864	23%	43.1%	41.8%
STM100724	YPS002032	19%	20.6%	39.7%
STM100866	BPT101006	33%	97.9%	91.7%
STM100866	BCE100250	33%	52.9%	98.0%
STM100866	BFU108046	30%	97.2%	99.7%
STM100866	BFU106928	32%	95.5%	94.2%
STM100866	BFU101670	31%	95.8%	97.1%
STM100866	BFU102611	33%	97.2%	94.9%
STM100866	BFU110913	33%	95.2%	95.9%
STM100866	BFU114233	33%	96.2%	97.3%
STM100866	BMA106862	28%	95.8%	92.0%
STM100866	CDP100121	25%	97.6%	96.4%
STM100866	EBC102104	73%	98.6%	98.3%
STM100866	EFA200698	55%	97.6%	95.6%
STM100866	ECO100606	63%	99.7%	94.5%
STM100866	HIN100022	61%	100%	99.7%
STM100866	KPN201811	89%	100%	100%
STM100866	MCA100880	23%	95.5%	97.0%
STM100866	MAV100316	30%	97.9%	95.2%
STM100866	MBV101161	31%	88.2%	86.8%
STM100866	MTU202461	31%	88.2%	86.8%
STM100866	PRT106096	26%	96.9%	97.5%
STM100866	PAE200882	30%	97.6%	97.5%
STM100866	PPU100881	28%	30.1%	11.6%
STM100866	SPA101729	96%	92.7%	100%
STM100866	STY101151	99%	100%	100%
STM100866	STM100866	100%	100%	100%
STM100866	SMU100150	56%	99.0%	95.7%
STM100866	SPY200907	57%	99.0%	97.3%
STM100866	VCH100784	65%	98.6%	96.6%
STM100866	YPS002471	30%	97.9%	96.1%
STM101115	BAN112350	30%	33.4%	40.1%
STM101115	BCE100786	33%	27.5%	28.5%
STM101115	BFU102061	27%	36.4%	39.9%
STM101115	BMA109995	30%	35.2%	35.3%
STM101115	CAC101453	33%	88.5%	81.3%
STM101115	CBO101891	30%	31.1%	33.8%
STM101115	CDF103042	31%	25.1%	48.3%
STM101115	CDP100635	25%	76.3%	82.6%
STM101115	EBC103957	84%	97.6%	100%
STM101115	EFA201896	22%	30.5%	33.6%
STM101115	ECO103737	81%	99.4%	98.8%
STM101115	HIN100626	30%	93.8%	98.4%
STM101115	KPN303520	81%	97.0%	99.4%
STM101115	LMO101007	29%	29.0%	32.4%
STM101115	MAV102145	25%	39.9%	47.3%
STM101115	MBV102643	28%	39.9%	47.0%
STM101115	MLP101539	23%	83.7%	92.8%
STM101115	MTU200182	28%	39.9%	40.6%
STM101115	MPN100368	48%	10.4%	13.1%
STM101115	PMU101631	28%	93.5%	97.5%
STM101115	PRT104544	48%	95.9%	97.3%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
STM101115	PAE203299	28%	28.4%	30.4%
STM101115	PPU108432	30%	28.4%	30.6%
STM101115	SPA101172	97%	100%	100%
STM101115	STY102009	99%	100%	100%
STM101115	STM101115	100%	100%	100%
STM101115	SAU801303	30%	44.4%	48.0%
STM101115	SEP201929	29%	35.2%	39.3%
STM101115	SHA102823	29%	29.9%	31.9%
STM101115	VCH100134	37%	93.8%	92.9%
STM101115	YPS001338	57%	97.6%	97.9%
STM101116	BAN105201	25%	87.6%	89.6%
STM101116	BAN104169	29%	90.2%	89.0%
STM101116	BBU100068	31%	97.7%	96.2%
STM101116	BCE113428	43%	30.5%	90%
STM101116	BFU104191	37%	99.2%	97.1%
STM101116	BMA100853	37%	99.2%	96.0%
STM101116	CPN200343	24%	89.5%	88.8%
STM101116	CTR200373	21%	89.8%	88.6%
STM101116	CAC101546	26%	90.2%	90.2%
STM101116	CAC100913	27%	88.3%	86.9%
STM101116	CAC101334	27%	90.2%	90%
STM101116	CBO101559	24%	89.8%	90.3%
STM101116	CDF102915	26%	89.5%	87.9%
STM101116	EBC103959	89%	99.6%	97.8%
STM101116	EFA202119	26%	90.6%	91.0%
STM101116	EFM200109	29%	90.2%	91.0%
STM101116	ECO103738	90%	57.5%	89.5%
STM101116	HIN100576	42%	98.9%	97.1%
STM101116	KPN303521	81%	99.6%	99.6%
STM101116	LMO102621	27%	90.6%	91.3%
STM101116	MAV103247	25%	98.9%	98.5%
STM101116	MBV102452	26%	87.2%	87.2%
STM101116	MLP100060	26%	96.6%	92.9%
STM101116	MTU203758	26%	87.2%	87.2%
STM101116	MPN100414	27%	80.5%	79.7%
STM101116	NGO100507	38%	34.2%	80.7%
STM101116	PMU101629	40%	98.9%	97.1%
STM101116	PRT105543	62%	99.2%	99.2%
STM101116	SPA101171	99%	75.2%	100%
STM101116	STY102007	100%	100%	100%
STM101116	STM101116	100%	100%	100%
STM101116	SAU801331	27%	98.5%	98.5%
STM101116	SEP200102	28%	90.6%	90.1%
STM101116	SHA100656	26%	98.1%	95.6%
STM101116	SMU100748	27%	98.5%	100%
STM101116	SPN400263	30%	98.9%	98.9%
STM101116	SPY201336	28%	98.9%	98.9%
STM101116	TPA100287	25%	89.1%	88.4%
STM101116	UUR100174	26%	88.7%	88.0%
STM101116	VCH100133	49%	97.4%	94.9%
STM101116	YPS001335	73%	99.6%	98.5%
STM101278	EBC107120	29%	17.1%	60.9%
STM101278	NME105091	31%	15.2%	17.7%
STM101278	PSY106217	25%	53.1%	52.6%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
STM101278	STM101278	100%	100%	100%
STM101278	SEP200437	28%	24.6%	34.2%
STM101278	SEP200202	28%	24.6%	34.2%
STM101278	SEP200828	28%	24.6%	34.2%
STM101278	SEP204358	36%	12.4%	36.5%
STM101278	SEP200771	28%	24.6%	34.2%
STM101278	SEP201864	28%	24.6%	34.2%
STM101278	SEP200165	28%	24.6%	34.2%
STM101278	SEP201913	28%	24.6%	34.2%
STM101278	SEP200187	28%	24.6%	34.2%
STM101278	SEP200347	28%	24.6%	34.2%
STM101278	SEP203770	28%	24.6%	34.2%
STM101278	SEP200222	28%	24.6%	34.2%
STM101923	BFR104413	56%	97.8%	95.5%
STM101923	BPT102042	44%	96.9%	95.0%
STM101923	BCE112542	59%	97.8%	97.8%
STM101923	BFU104304	58%	96.9%	93.0%
STM101923	BMA105070	57%	97.8%	97.4%
STM101923	CAC101675	30%	89.3%	84.0%
STM101923	CBO102946	31%	87.5%	83.9%
STM101923	CDF104256	28%	90.2%	89.3%
STM101923	CDP100150	30%	98.7%	97.9%
STM101923	EBC101616	89%	38.7%	100%
STM101923	EFA202385	30%	91.9%	88.7%
STM101923	ECO101796	90%	100%	92.5%
STM101923	KPN301541	79%	99.3%	99.6%
STM101923	MAV103783	30%	88.8%	74.1%
STM101923	MBV105656	29%	88.2%	77.6%
STM101923	MTU202299	29%	88.2%	75.6%
STM101923	PAE200245	30%	89.7%	82.8%
STM101923	SPA100492	98%	98.9%	100%
STM101923	STM101923	100%	100%	100%
STM101923	VCH101573	28%	88.6%	84.9%
STM101923	YPS000541	69%	98.9%	99.3%
STM101955	SPA104151	96%	100%	100%
STM101955	STM101955	100%	100%	100%
STM102011	STM102011	100%	100%	100%
STM102089	ABA104944	29%	76.3%	81.9%
STM102089	BAN113702	44%	45.9%	72.6%
STM102089	BAN111016	34%	98.2%	100%
STM102089	BAN102075	36%	93.0%	90.5%
STM102089	BAN106548	36%	100%	99.4%
STM102089	BAN111025	34%	100%	100%
STM102089	BFR100859	25%	46.2%	35.5%
STM102089	BFR102551	26%	49.8%	38.2%
STM102089	BPT101117	35%	98.2%	91.8%
STM102089	BCE102679	33%	98.8%	92.7%
STM102089	BFU105518	35%	86.6%	79.7%
STM102089	BMA107904	35%	98.8%	100%
STM102089	CJU100150	33%	92.7%	93.1%
STM102089	CPN200938	28%	20.4%	18.7%
STM102089	CAC101752	32%	98.8%	100%
STM102089	CBO102636	31%	98.8%	99.7%
STM102089	CDF101204	30%	98.8%	100%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
STM102089	CDP101688	31%	86.0%	98.0%
STM102089	EBC102210	89%	100%	100%
STM102089	EFA202347	35%	98.8%	100%
STM102089	ECO100757	92%	100%	100%
STM102089	HIN101641	60%	99.4%	96.7%
STM102089	HPY100755	28%	98.8%	99.7%
STM102089	KPN303579	87%	100%	100%
STM102089	LMO102857	33%	98.8%	99.1%
STM102089	MCA103672	44%	99.1%	89.5%
STM102089	MAV105594	35%	95.1%	87.4%
STM102089	MBV102851	33%	92.4%	83.4%
STM102089	MTU203069	33%	92.4%	85.5%
STM102089	PMU100625	61%	98.8%	96.1%
STM102089	PRT104379	72%	99.1%	99.4%
STM102089	PAE201504	35%	99.4%	99.1%
STM102089	PPU106301	36%	93.3%	92.2%
STM102089	PSY105427	35%	98.2%	99.7%
STM102089	SPA102732	99%	100%	100%
STM102089	STY102330	100%	100%	100%
STM102089	STM102089	100%	100%	100%
STM102089	SAU802268	33%	100%	100%
STM102089	SEP200319	32%	100%	100%
STM102089	SHA101565	31%	100%	100%
STM102089	SPN400676	25%	48.6%	47.9%
STM102089	VCH101005	64%	100%	98.5%
STM102089	YPS002149	76%	99.1%	99.4%
STM102090	BAN104975	42%	84.7%	85.8%
STM102090	BFR102256	23%	81.2%	38.5%
STM102090	BPT101121	52%	91.8%	90.2%
STM102090	EBC102209	91%	100%	100%
STM102090	ECO100758	95%	100%	100%
STM102090	KPN303581	92%	100%	100%
STM102090	LMO101377	38%	87.1%	91.4%
STM102090	MCA101697	58%	95.9%	86.2%
STM102090	MBV100621	31%	81.8%	76.8%
STM102090	MLP100125	33%	81.2%	76.2%
STM102090	MTU200973	31%	81.8%	76.8%
STM102090	PAE203912	58%	97.1%	89.2%
STM102090	PAE203027	58%	95.9%	91.1%
STM102090	PPU101063	57%	97.6%	96.5%
STM102090	PSY109125	54%	99.4%	94.4%
STM102090	SPA102733	99%	100%	100%
STM102090	STY102331	100%	100%	100%
STM102090	STM102090	100%	100%	100%
STM102090	SAU802275	45%	85.3%	87.5%
STM102090	SEP200340	42%	85.9%	85.2%
STM102090	SHA101559	46%	84.1%	84.8%
STM102090	SMU101232	25%	56.5%	21.3%
STM102090	SPN401756	29%	51.8%	19.4%
STM102090	VCH101006	63%	98.8%	98.8%
STM102366	STM102366	100%	100%	100%
STM102401	BPT105648	41%	100%	42.0%
STM102401	BCE107576	40%	99.4%	73.4%
STM102401	BFU113002	41%	45.3%	33.2%



Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
STM102401	BMA104672	37%	99.4%	47.0%
STM102401	MAV108057	34%	99.4%	76.8%
STM102401	MBV105368	35%	99.4%	80.3%
STM102401	MTU409357	35%	99.4%	80.3%
STM102401	PAE109984	40%	99.4%	89.1%
STM102401	PSY100104	42%	95.0%	95.6%
STM102401	STM102401	100%	100%	100%
STM102419	BPT105323	33%	92.8%	49.0%
STM102419	PAE111918	43%	64.0%	51.8%
STM102419	PSY103395	35%	91.9%	54.3%
STM102419	SPA106234	100%	100%	82.2%
STM102419	STM102419	100%	100%	100%
STM102422	CDP102587	30%	73.9%	35.2%
STM102422	EBC105759	91%	48.9%	100%
STM102422	KPN206188	95%	48.9%	100%
STM102422	STM102422	100%	100%	100%
STM102449	BFU104448	49%	99.2%	67.8%
STM102449	MBV100454	32%	99.2%	80%
STM102449	MTU411274	32%	99.2%	80%
STM102449	FRT105025	61%	48%	88.6%
STM102449	PAE111567	48%	23.2%	96.7%
STM102449	STM102449	100%	100%	100%
STM102449	SPY103286	52%	38.4%	98.0%
STM102672	ABA105833	40%	96.4%	99.4%
STM102672	BAN101367	20%	65.6%	68.2%
STM102672	BPT101286	48%	97.5%	99.2%
STM102672	BBU100809	25%	96.6%	97.5%
STM102672	BCE109401	52%	97.1%	99.6%
STM102672	BFU100360	52%	97.1%	99.6%
STM102672	BMA106132	50%	98.1%	81.1%
STM102672	CJU100740	31%	86.3%	92.5%
STM102672	CPN200015	23%	82.6%	78.1%
STM102672	CTR200901	22%	88.4%	83.6%
STM102672	CAC103368	27%	82.8%	82.5%
STM102672	CBO100146	25%	84.4%	93.0%
STM102672	CDF100314	28%	23.1%	87.4%
STM102672	CDF100311	26%	70.2%	91.0%
STM102672	CDF103058	27%	95.6%	95.6%
STM102672	CDP101613	25%	77.9%	37.2%
STM102672	EBC101202	91%	97.5%	100%
STM102672	EFA201075	21%	72.5%	75.7%
STM102672	EFM201627	23%	43.9%	42.0%
STM102672	ECO101042	94%	97.3%	99.8%
STM102672	HIN100943	39%	93.5%	98.4%
STM102672	HPY100871	28%	83.8%	98.0%
STM102672	KPN302527	90%	100%	100%
STM102672	LPN102528	45%	87.0%	98.7%
STM102672	LMO100585	23%	36.6%	33.5%
STM102672	MCA103523	43%	95.4%	97.7%
STM102672	MBV102833	27%	79.8%	37.1%
STM102672	MLP101595	27%	91.6%	40.0%
STM102672	MTU203855	27%	80.7%	36.7%
STM102672	NGO101881	64%	96.9%	99.4%
STM102672	NME202045	63%	96.9%	99.4%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
STM102672	PMU101660	40%	93.5%	98.4%
STM102672	PRT101541	76%	97.1%	99.6%
STM102672	PAE204560	76%	97.1%	99.4%
STM102672	PPU112503	71%	97.1%	99.4%
STM102672	PSY102688	74%	93.5%	99.4%
STM102672	SPA100422	94%	100%	100%
STM102672	STY102506	99%	94.8%	100%
STM102672	STM102672	100%	100%	100%
STM102672	SAU801754	20%	40.8%	38.9%
STM102672	SEP202115	20%	40.3%	38.5%
STM102672	SHA101207	19%	78.4%	75.0%
STM102672	SMU100383	21%	40.8%	38.2%
STM102672	SPN401382	24%	26.0%	25.2%
STM102672	TPA100512	25%	89.5%	88.4%
STM102672	VCH100667	41%	97.9%	99.0%
STM102672	YPS003371	85%	97.3%	99.8%
STM102789	ABA101658	46%	92.6%	91.1%
STM102789	BAN113740	23%	69.7%	78.7%
STM102789	BAN102304	24%	87.7%	91.5%
STM102789	BFR10945	29%	87.7%	82.8%
STM102789	BPT100037	45%	96.7%	89.4%
STM102789	BCE110334	52%	91.0%	62.7%
STM102789	BFU105764	51%	91.0%	48.1%
STM102789	BMA107802	48%	91.0%	61.3%
STM102789	CJU100229	41%	86.1%	89.0%
STM102789	CAC100848	21%	89.3%	47.0%
STM102789	CBO101544	27%	92.6%	48.7%
STM102789	CDF103965	27%	88.5%	45.2%
STM102789	EBC104206	81%	100%	100%
STM102789	EFA200393	24%	87.7%	80.5%
STM102789	EFM201127	27%	83.6%	77.9%
STM102789	ECO103936	86%	99.2%	99.2%
STM102789	HIN100318	45%	93.4%	96.6%
STM102789	HPY100691	41%	88.5%	84.4%
STM102789	KPN306685	80%	28.7%	97.2%
STM102789	KPN306067	78%	34.4%	97.7%
STM102789	KPN301864	83%	99.2%	99.2%
STM102789	LMO102033	29%	86.1%	80.2%
STM102789	NGO101285	40%	88.5%	85.0%
STM102789	NME201605	40%	87.7%	84.3%
STM102789	PMU101864	50%	93.4%	96.6%
STM102789	PRT101484	64%	99.2%	98.4%
STM102789	PAE203601	50%	91.8%	91.1%
STM102789	PPU101143	50%	91.8%	92.6%
STM102789	PSY104412	45%	91.8%	92.6%
STM102789	SPA103524	99%	100%	100%
STM102789	STY101940	100%	100%	100%
STM102789	STM102789	100%	100%	100%
STM102789	SAU801569	28%	90.2%	96.5%
STM102789	SEP201025	31%	81.1%	83.9%
STM102789	SHA101649	34%	57.4%	97.2%
STM102789	SMU101129	38%	77.0%	70.1%
STM102789	SPN400870	34%	82.0%	77.9%
STM102789	SPY200341	31%	91.0%	85.9%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
STM102789	VCH103435	58%	95.9%	99.2%
STM102789	YPS002785	67%	98.4%	97.6%
STM102835	BAN100006	22%	40.4%	67.4%
STM102835	BFR106009	25%	50.8%	36.6%
STM102835	BPT100671	23%	68.2%	40.9%
STM102835	BCE102211	26%	1.3%	30.4%
STM102835	BCE104738	24%	53.1%	59.1%
STM102835	BCE103017	25%	40.6%	90.6%
STM102835	BCE110445	26%	35.6%	94.0%
STM102835	BCE103623	26%	1.6%	96.7%
STM102835	BCE110366	23%	13.5%	38.0%
STM102835	BCE101250	24%	61.2%	55.7%
STM102835	BCE100403	25%	3.8%	94.4%
STM102835	BFU113446	21%	12.9%	55.0%
STM102835	BFU106257	21%	16.8%	73.7%
STM102835	BFU112017	23%	53.6%	73.3%
STM102835	BFU104375	23%	53.6%	42.8%
STM102835	BFU106700	23%	46.7%	39.2%
STM102835	BFU112565	21%	69.6%	66.3%
STM102835	BFU109807	23%	53.3%	69.1%
STM102835	BFU108657	22%	54.3%	87.1%
STM102835	BMA108282	26%	58.6%	88.4%
STM102835	CJU101583	22%	16.3%	57.3%
STM102835	CJU100590	22%	61.9%	1.7%
STM102835	CTR200684	18%	40.1%	78.3%
STM102835	CBO102757	30%	3.9%	10.6%
STM102835	CDF101661	31%	8.4%	12.5%
STM102835	CDP101991	22%	13.8%	24.4%
STM102835	EFA201471	23%	63.7%	34.5%
STM102835	EFM200844	23%	57.8%	25.0%
STM102835	ECO101376	47%	12.6%	66.5%
STM102835	HPY200552	22%	40.9%	61.1%
STM102835	HPY200273	20%	48.7%	23.6%
STM102835	HPY200815	19%	41.3%	64.1%
STM102835	HPY200852	21%	1.4%	6.3%
STM102835	KPN206267	23%	9.4%	28.1%
STM102835	MAV104123	30%	5.6%	60.7%
STM102835	MBV102102	23%	63.2%	14.2%
STM102835	MTU200354	21%	56.0%	88.8%
STM102835	MTU200303	23%	17.8%	78.1%
STM102835	NME200634	21%	47.9%	41.9%
STM102835	SPA103181	85%	35.0%	97.7%
STM102835	STM102835	100%	100%	100%
STM102835	SAU800561	24%	12.7%	16.7%
STM102835	SAU800562	22%	30.5%	2.5%
STM102835	SAU800563	26%	10.9%	15.7%
STM102835	SAU800811	21%	12.4%	34.5%
STM102835	SAU802630	27%	8.3%	16.1%
STM102835	SEP100151	29%	7.2%	19.9%
STM102835	SEP100719	25%	7.7%	2.1%
STM102835	SHA100562	19%	79.5%	4.8%
STM102835	SMU101698	19%	49.7%	91.5%
STM102835	SPY201332	21%	7.1%	32.8%
STM102835	VCH101430	20%	22.5%	81.9%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
STM103180	BFR14231	25%	13.8%	43.0%
STM103180	BCE105486	36%	92.0%	95.5%
STM103180	EBC101869	50%	79.4%	98.6%
STM103180	ECO102296	58%	65.4%	100%
STM103180	PRT104898	38%	90.4%	98.7%
STM103180	PRT104430	46%	93.8%	93.3%
STM103180	PRT100691	48%	96.8%	97.2%
STM103180	SPA103176	90%	93.9%	100%
STM103180	STY103516	53%	98.9%	97.2%
STM103180	STM103180	100%	100%	100%
STM103180	YPS002772	44%	92.5%	97.5%
STM103235	CAC102031	25%	13.4%	43.5%
STM103235	CBO103445	29%	9.5%	35.5%
STM103235	ECO103932	20%	51.5%	84.6%
STM103235	KPN301199	31%	12.1%	13.0%
STM103235	SPA103638	95%	76.5%	100%
STM103235	STM103235	100%	100%	100%
STM103235	YPS000110	32%	12.1%	13.0%
STM103247	BCE107517	29%	82.1%	70.2%
STM103247	BMA103289	32%	58.0%	58.2%
STM103247	KPN207172	50%	10.1%	58.3%
STM103247	PPU107587	30%	79.8%	76%
STM103247	PSY106811	32%	81.8%	84.1%
STM103247	SPA106549	94%	14.6%	59.1%
STM103247	STM103247	100%	100%	100%
STM103274	BFU113111	30%	27.2%	15.5%
STM103274	STM103274	100%	100%	100%
STM103418	EBC106113	76%	98.4%	52.1%
STM103418	STM103418	100%	100%	100%
STM103506	KPN200136	32%	27.7%	23.2%
STM103506	SPA105363	92%	11.8%	65.1%
STM103506	STM103506	100%	100%	100%
STM103802	BMA103619	25%	42.7%	73.4%
STM103802	BMA106999	27%	87.2%	94.5%
STM103802	HPY201193	24%	11.9%	69.1%
STM103802	STM103802	100%	100%	100%
STM103805	ABA102812	33%	16.9%	22.1%
STM103805	ABA105767	23%	62.4%	49.5%
STM103805	ABA106133	25%	63.4%	49.3%
STM103805	BFR100630	24%	29.9%	36.1%
STM103805	BCE104450	37%	39.2%	94.1%
STM103805	BCE111308	33%	80.9%	94.6%
STM103805	BFU107929	43%	79.0%	88.5%
STM103805	BFU100791	45%	78.7%	82.5%
STM103805	BFU102584	45%	80.4%	90.2%
STM103805	BMA104805	46%	71.3%	78.0%
STM103805	EBC102287	37%	80.2%	67.4%
STM103805	ECO200602	33%	74.3%	84.2%
STM103805	ECO202018	32%	77.5%	76.6%
STM103805	ECO200236	32%	78.7%	78.0%
STM103805	KPN305296	25%	69.4%	61.5%
STM103805	MBV100192	36%	12.2%	27.2%
STM103805	MTU202980	35%	11.2%	20.3%
STM103805	PKT100729	28%	77.0%	77.7%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
STM103805	PRT101866	27%	95.9%	91.5%
STM103805	PAE202683	38%	77.6%	77.4%
STM103805	PAE200091	37%	83.8%	94.2%
STM103805	PAE200095	39%	81.6%	96.0%
STM103805	PPU110472	34%	80.9%	95.3%
STM103805	PSY102082	32%	83.8%	90%
STM103805	SPA102886	79%	97.7%	100%
STM103805	STY104093	99%	100%	100%
STM103805	STM103805	100%	100%	100%
STM103805	VCH102859	27%	82.7%	60.0%
STM103805	VCH101395	30%	79.8%	50.5%
STM103805	VCH102754	31%	78.5%	83.4%
STM103805	YPS002256	38%	85.7%	72.9%
STM103815	STM103815	100%	100%	100%
STM103908	BCE114708	28%	99.6%	99.3%
STM103908	BFU100174	28%	51.3%	97.9%
STM103908	BFU100103	29%	99.6%	99.3%
STM103908	BMA108675	29%	99.6%	99.0%
STM103908	EBC102778	81%	82.2%	94.6%
STM103908	ECO102028	88%	100%	95.5%
STM103908	KPN301730	84%	100%	95.3%
STM103908	PRT101397	53%	100%	100%
STM103908	PAE204868	29%	97.8%	96.0%
STM103908	PPU100478	27%	97.8%	95.5%
STM103908	PSY101023	29%	97.6%	95.7%
STM103908	SPA100063	86%	68.7%	100%
STM103908	STY104171	99%	100%	100%
STM103908	STM103908	100%	100%	100%
STM103908	VCH101366	54%	100%	100%
STM103908	YPS003105	71%	100%	100%
STM103938	ABA104340	34%	47.1%	38.6%
STM103938	BCE100985	29%	51.9%	43.2%
STM103938	BFU111670	34%	47.1%	37.4%
STM103938	BMA108444	33%	51.9%	42.9%
STM103938	EBC101816	63%	99.0%	98.2%
STM103938	ECO102066	80%	100%	64.0%
STM103938	KPN304485	70%	99.0%	98.1%
STM103938	PPU110423	34%	84.6%	83.3%
STM103938	PSY105412	32%	48.1%	51.5%
STM103938	SPA103081	100%	100%	95.4%
STM103938	STY104192	100%	100%	100%
STM103938	STM103938	100%	100%	100%
STM104133	BPT105409	27%	81.1%	76.7%
STM104133	BCE103874	31%	67.7%	49.1%
STM104133	BMA103613	24%	87.6%	98.4%
STM104133	STM104133	100%	100%	100%
STM104223	EFA203072	34%	13.4%	11.1%
STM104223	MLP101303	26%	24.4%	26.9%
STM104223	STM104223	100%	100%	100%
STM104237	STM104237	100%	100%	100%
STM104276	EBC106188	44%	98.6%	84.5%
STM104276	KPN109099	52%	78.6%	75.2%
STM104276	STM104276	100%	100%	100%
STM104686	BCE110467	45%	98.6%	100%

Query LocusID	Homolog LocusID	Identity	Query Coverage	Homolog Coverage
STM104686	BFU102583	27%	81.8%	78.5%
STM104686	BMA102061	28%	86.5%	72.5%
STM104686	EBC104891	25%	89.2%	89.0%
STM104686	KPN107511	36%	38.5%	58.1%
STM104686	PRT103753	26%	96.6%	98.6%
STM104686	PAE200094	33%	41.9%	43.8%
STM104686	PPU110480	40%	94.6%	80.3%
STM104686	STY104879	100%	100%	100%
STM104686	STM104686	100%	100%	100%
STM104686	YPS004196	35%	94.6%	89.5%

The data in Table IV demonstrates the methods described herein identified genes required for proliferation in several species which share homology.

#### EXAMPLE 9

##### Identification of Genes and their Corresponding Operons Affected by Antisense Inhibition

5 Once the genes involved in *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium*  
10 *botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus*  
15 *mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* proliferation are identified as described above, the operons in which these genes lie may be identified by comparison with known microbial genomes. Since bacterial genes are  
20 transcribed in a polycistronic manner, the antisense inhibition of a single gene in an operon might affect the expression of all the other genes on the operon or the genes downstream from the single gene identified. Accordingly, each of the genes contained within an operon may be analyzed for their effect on proliferation.

Operons are predicted by looking for all adjacent genes in a genomic region that lie in the  
25 same orientation with no large noncoding gaps in between. First, full-length ORFs complementary to the antisense molecules are identified as described above. Adjacent ORFs are then identified and their relative orientation determined either by directly analyzing the genomic sequences surrounding the ORFs complementary to the antisense clones or by extracting adjacent ORFs from the collection obtained through whole genome ORF analysis described above followed by ORF  
30 alignment. Operons predicted in this way may be confirmed by comparison to the arrangement of the homologous nucleic acids in the *Bacillus subtilis* complete genome sequence, as reported by the genome database compiled at Institut Pasteur Subtilist Release R15.1 (June 24, 1999) which can be found at <http://bioweb.pasteur.fr/GenoList/Subtilist/>. The *Bacillus subtilis* genome is the only fully  
35 sequenced and annotated genome from a Gram positive microorganism, and appears to have a high level of similarity to *Staphylococcus aureus* both at the level of conservation of gene sequence and genomic organization including operon structure. Operons for *Salmonella typhimurium* and *Klebsiella pneumoniae* may be identified by comparison with *E. coli*, *Haemophilus*, or

*Pseudomonas* sequences. The *Pseudomonas aeruginosa* web site (<http://www.pseudomonas.com>) can also be used to help predict operon organization in this bacterium.

Extensive DNA sequences of *Salmonella typhimurium* are available through the Salmonella Genome Center (Washington University, St. Louis, MO) the Sanger Centre (United Kingdom) and the PathoSeq database (Incyte ). Annotation of some of the DNA sequences in some of the  
5 the aforementioned databases is lacking, but comparisons may be made to *E. coli* using tools such as BLASTX.

Public or proprietary databases may be used to analyzed *E. faecalis* sequences as well as sequences from the organisms listed above.

10 The analysis of the operons on which essential genes lie may be conducted for each of the sequences which are listed in Table IA which inhibit proliferation and the ORFs listed in Table IC. Once the full length ORFs and/or the operons containing them have been identified using the methods described above, they can be obtained from a genomic library by performing a PCR amplification using primers at each end of the desired sequence. Those skilled in the art will  
15 appreciate that a comparison of the ORFs to homologous sequences in other cells or microorganisms will facilitate confirmation of the start and stop codons at the ends of the ORFs.

In some embodiments, the primers may contain restriction sites which facilitate the insertion of the gene or operon into a desired vector. For example, the gene may be inserted into an expression vector and used to produce the proliferation-required protein as described below. Other  
20 methods for obtaining the full length ORFs and/or operons are familiar to those skilled in the art. For example, natural restriction sites may be employed to insert the full length ORFs and/or operons into a desired vector.

#### EXAMPLE 10

##### Identification of Individual Genes within an Operon Required for Proliferation

25 The following example illustrates a method for determining if a targeted gene within an operon is required for cell proliferation by replacing the targeted allele in the chromosome with an in-frame deletion of the coding region of the targeted gene.

Deletion inactivation of a chromosomal copy of a gene in *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*,  
30 *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*,



*Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* can be accomplished by integrative gene replacement. The principles of this method were described in Xia, M., et al. 1999 Plasmid 42:144-149 and Hamilton, C. M., et al 1989. *J. Bacteriol.* 171: 4617-4622. A similar gene disruption method is available for *Pseudomonas aeruginosa*, except the counter selectable marker is *sacB* (Schweizer, H. P., Klassen, T. and Hoang, T. (1996) *Mol. Biol. of Pseudomonas*. ASM press, 229-237. In this approach, a mutant allele of the targeted gene is constructed by way of an in-frame deletion and introduced into the chromosome using a suicide vector. This results in a tandem duplication comprising a deleted (null) allele and a wild type allele of the target gene. Cells in which the vector sequences have been deleted are isolated using a counter-selection technique. Removal of the vector sequence from the chromosomal insertion results in either restoration of the wild-type target sequence or replacement of the wild type sequence with the deletion (null) allele. *E. faecalis* genes can be disrupted using a suicide vector that contains an internal fragment to a gene of interest. With the appropriate selection this plasmid will homologously recombine into the chromosome (Nallapareddy, S. R., X. Qin, G. M. Weinstock, M. Hook, B. E. Murray. 2000. *Infect. Immun.* 68:5218-5224.

The resultant population of *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* colonies can then be evaluated to determine whether the target sequence is required for proliferation by PCR amplification of the affected target sequence. If the targeted gene is not required for proliferation, then PCR analysis will show that roughly equal numbers of colonies have retained either the wild-type or the mutant allele. If the targeted gene is required for proliferation, then only wild-type alleles will be recovered in the PCR analysis.

The method of cross-over PCR is used to generate the mutant allele by amplification of nucleotide sequences flanking but not including the coding region of the gene of interest, using specifically designed primers such that overlap between the resulting two PCR amplification

products allows them to hybridize. Further PCR amplification of this hybridization product using primers representing the extreme 5' and 3' ends can produce an amplification product containing an in-frame deletion of the coding region but retaining substantial flanking sequences.

For *Staphylococcus aureus*, this amplification product is subcloned into the suicide vector pSA3182 (Xia, M., et al. 1999 Plasmid 42:144-149, which is host-dependent for autonomous replication. This vector includes a *tetC* tetracycline-resistance marker and the origin of replication of the well-known *Staphylococcus aureus* plasmid pT181 (Mojumdar, M and Kahn, S.A., Characterisation of the Tetracycline Resistance Gene of Plasmid pT181, J. Bacteriol. 170: 5522 (1988). The vector lacks the *repC* gene which is required for autonomous replication of the vector at the pT181 origin. This vector can be propagated in a *Staphylococcus aureus* host strain such as SA3528, which expresses *repC* in trans. Once the amplified truncated target gene sequence is cloned and propagated in the pSA3182 vector, it can then be introduced into a *repC* minus strain such as RN4220 (Kreiswirth, B.N. et al., The Toxic Shock Syndrome Exotoxin Structural Gene is Not Detectably Transmitted by a Prophage, Nature 305:709-712 (1983), by electroporation with selection for tetracycline resistance. In this strain, the vector must integrate by homologous recombination at the targeted gene in the chromosome to impart drug resistance. This results in an inserted truncated copy of the allele, followed by pSA3182 vector sequence, and finally an intact and functional allele of the targeted gene.

Once a tetracycline resistant *Staphylococcus aureus* strain is isolated using the above technique and shown to include truncated and wild-type alleles of the targeted gene as described above, a second plasmid, pSA7592 (Xia, M., et al. 1999 Plasmid 42:144-149, is introduced into the strain by electroporation. This gene includes an erythromycin resistance gene and a *repC* gene that is expressed at high levels. Expression of *repC* in these transformants is toxic due to interference of normal chromosomal replication at the integrated pT181 origin of replication. This selects for strains that have removed the vector sequence by homologous recombination, resulting in either of two outcomes: The selected cells either possess a wild-type allele of the targeted gene or a gene in which the wild-type allele has been replaced by the engineered in-frame deletion of the truncated allele.

PCR amplification can be used to determine the genetic outcome of the above process in the resulting erythromycin resistant, tet sensitive transformant colonies. If the targeted gene is not required for cellular replication, then PCR evidence for both wild-type and mutant alleles will be found among the population of resultant transformants. However, if the targeted gene is required for cellular proliferation, then only the wild-type form of the gene will be evident among the resulting transformants.

Similarly, for *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia*

*pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diphtheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae,*  
 5 *Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae* or  
 10 *Yersinia pestis* the PCR products containing the mutant allele of the target sequence may be introduced into an appropriate knockout vector and cells in which the wild type target has been disrupted are selected using the appropriate methodology.

The above methods have the advantage that insertion of an in-frame deletion mutation is far less likely to cause downstream polar effects on genes in the same operon as the targeted gene.  
 15 However, it will be appreciated that other methods for disrupting *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diphtheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella*  
 20 *multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae* or *Yersinia pestis* genes which are familiar to those skilled in the art may also be used.  
 25 Each gene in the operon may be disrupted using the methodology above to determine  
 30 whether it is required for proliferation.

#### EXAMPLE 11

Expression of the Proteins Encoded by Genes Identified as Required for *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*,  
 35 *Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium*

diphtheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Yersinia pestis Proliferation

The following is provided as one exemplary method to express the proliferation-required proteins identified as described above. The proliferation-required proteins may be expressed using any of the bacterial, insect, yeast, or mammalian expression systems known in the art. In some embodiments, the proliferation-required proteins encoded by the identified nucleotide sequences described above (including the proteins of SEQ ID NOs.: 42398-78581 encoded by the nucleic acids of SEQ ID NOs.: 6214-42397 are expressed using expression systems designed either for *E. coli* or for *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis*. First, the initiation and termination codons for the gene are identified. If desired, methods for improving translation or expression of the protein are well known in the art. For example, if the nucleic acid encoding the polypeptide to be expressed lacks a methionine codon to serve as the initiation site, a strong Shine-Delgarno sequence, or a stop codon, these nucleotide sequences can be added. Similarly, if the identified nucleic acid lacks a transcription termination signal, this nucleotide sequence can be added to the construct by, for example, splicing out such a sequence from an appropriate donor sequence. In addition, the coding sequence may be operably linked to a strong constitutive promoter or an inducible promoter if desired. The identified nucleic acid or portion thereof encoding the polypeptide to be expressed is obtained by, for example, PCR from the bacterial expression vector or genome using oligonucleotide primers complementary to the identified nucleic acid or portion thereof and containing restriction endonuclease sequences appropriate for inserting the coding sequences into the vector such that the coding sequences can be

expressed from the vector's promoter. Alternatively, other conventional cloning techniques may be used to place the coding sequence under the control of the promoter. In some embodiments, a termination signal may be located downstream of the coding sequence such that transcription of the coding sequence ends at an appropriate position.

5 Several expression vector systems for protein expression in *E. coli* are well known and available to those knowledgeable in the art. The coding sequence may be inserted into any of these vectors and placed under the control of the promoter. The expression vector may then be transformed into DH5 $\alpha$  or some other *E. coli* strain suitable for the over expression of proteins.

Alternatively, an expression vector encoding a protein required for proliferation of  
 10 *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*,  
 15 *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* may be introduced into *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*,  
 25 *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*,  
 30 *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*,  
 35 *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis*. Protocols for introducing nucleic acids into these organisms are well known in the art. For example, the protocols described in J.C.Lee "Electroporation of Staphylococci" from Methods in Molecular Biology vol 47: Electroporation Protocols for Microorganisms Edited by :

J.A. Nickoloff Humana Press Inc., Totowa, NJ. pp209-216, may be used to introduce nucleic acids into *Staphylococcus aureus*. Nucleic acids may also be introduced into *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* or *Enterococcus faecalis* using methods familiar to those skilled in the art. Positive transformants are selected after growing the transformed cells on  
5 plates containing an antibiotic to which the vector confers resistance. In one embodiment, *Staphylococcus aureus* is transformed with an expression vector in which the coding sequence is operably linked to the T5 promoter containing a xylose operator such that expression of the encoded protein is inducible with xylose.

In one embodiment, the protein is expressed and maintained in the cytoplasm as the native  
10 sequence. In an alternate embodiment, the expressed protein can be modified to include a protein tag that allows for differential cellular targeting, such as to the periplasmic space of Gram negative or Gram positive expression hosts or to the exterior of the cell (i.e., into the culture medium). In some embodiments, the osmotic shock cell lysis method described in Chapter 16 of **Current Protocols in Molecular Biology**, Vol. 2, (Ausubel, et al., Eds.) John Wiley & Sons, Inc. (1997) may  
15 be used to liberate the polypeptide from the cell. In still another embodiment, such a protein tag could also facilitate purification of the protein from either fractionated cells or from the culture medium by affinity chromatography. Each of these procedures can be used to express a proliferation-required protein.

Expressed proteins, whether in the culture medium or liberated from the periplasmic space or  
20 the cytoplasm, are then purified or enriched from the supernatant using conventional techniques such as ammonium sulfate precipitation, standard chromatography, immunoprecipitation, immunochromatography, size exclusion chromatography, ion exchange chromatography, and HPLC. Alternatively, the polypeptide may be secreted from the host cell in a sufficiently enriched or pure state in the supernatant or growth media of the host cell to permit it to be used for its intended purpose  
25 without further enrichment. The purity of the protein product obtained can be assessed using techniques such as SDS PAGE, which is a protein resolving technique well known to those skilled in the art. Coomassie, silver staining or staining with an antibody are typical methods used to visualize the protein of interest.

Antibodies capable of specifically recognizing the protein of interest can be generated using  
30 synthetic peptides using methods well known in the art. See, **Antibodies: A Laboratory Manual**, (Harlow and Lane, Eds.) Cold Spring Harbor Laboratory (1988). For example, 15-mer peptides having an amino acid sequence encoded by the appropriate identified gene sequence of interest or portion thereof can be chemically synthesized. The synthetic peptides are injected into mice to generate antibodies to the polypeptide encoded by the identified nucleic acid sequence of interest or portion  
35 thereof. Alternatively, samples of the protein expressed from the expression vectors discussed above can be purified and subjected to amino acid sequencing analysis to confirm the identity of the recombinantly expressed protein and subsequently used to raise antibodies. An Example describing in detail the generation of monoclonal and polyclonal antibodies appears in Example 12.

The protein encoded by the identified nucleic acid of interest or portion thereof can be purified using standard immunochromatography techniques. In such procedures, a solution containing the secreted protein, such as the culture medium or a cell extract, is applied to a column having antibodies against the secreted protein attached to the chromatography matrix. The secreted protein is allowed to  
 5 bind the immunochromatography column. Thereafter, the column is washed to remove non-specifically bound proteins. The specifically-bound secreted protein is then released from the column and recovered using standard techniques. These procedures are well known in the art.

In an alternative protein purification scheme, the identified nucleic acid of interest or portion thereof can be incorporated into expression vectors designed for use in purification schemes employing  
 10 chimeric polypeptides. In such strategies the coding sequence of the identified nucleic acid of interest or portion thereof is inserted in-frame with the gene encoding the other half of the chimera. The other half of the chimera can be maltose binding protein (MBP) or a nickel binding polypeptide encoding sequence. A chromatography matrix having maltose or nickel attached thereto is then used to purify the chimeric protein. Protease cleavage sites can be engineered between the MBP gene or the nickel  
 15 binding polypeptide and the identified expected gene of interest, or portion thereof. Thus, the two polypeptides of the chimera can be separated from one another by protease digestion.

One useful expression vector for generating maltose binding protein fusion proteins is pMAL (New England Biolabs), which encodes the *malE* gene. In the pMal protein fusion system, the cloned gene is inserted into a pMal vector downstream from the *malE* gene. This results in the expression of  
 20 an MBP-fusion protein. The fusion protein is purified by affinity chromatography. These techniques as described are well known to those skilled in the art of molecular biology.

#### EXAMPLE 12

Production of an Antibody to an isolated *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter*  
 25 *baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria*  
 30 *monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus*  
 35 *pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* Protein

Substantially pure protein or polypeptide (including one of the polypeptides of SEQ ID NOs.: 42398-78581) is isolated from the transformed cells as described in Example 11. The concentration of

protein in the final preparation is adjusted, for example, by concentration on a 10,000 molecular weight cut off AMICON filter device (Millipore, Bedford, MA), to the level of a few micrograms/ml. Monoclonal or polyclonal antibody to the protein can then be prepared as follows:

#### **Monoclonal Antibody Production by Hybridoma Fusion**

5            Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler, G. and Milstein, C., *Nature* 256:495 (1975) or any of the well-known derivative methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein or peptides derived therefrom over a period of a few weeks. The mouse is then sacrificed, and the antibody-producing cells of the  
10        spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells are destroyed by growth of the system on selective medium comprising aminopterin (HAT medium). The successfully-fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay  
15        procedures, such as ELISA, as described by Engvall, E., "Enzyme immunoassay ELISA and EMIT," *Meth. Enzymol.* 70:419 (1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. et al. **Basic Methods in Molecular Biology** Elsevier, New York. Section 21-2.

#### **20            Polyclonal Antibody Production by Immunization**

          Polyclonal antiserum containing antibodies to heterogeneous epitopes of a single protein or a peptide can be prepared by immunizing suitable animals with the expressed protein or peptides derived therefrom described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the  
25        host species. For example, small molecules tend to be less immunogenic than larger molecules and can require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. et al. *J. Clin. Endocrinol. Metab.*  
30        33:988-991 (1971).

          Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony, O. et al., Chap. 19 in: **Handbook of Experimental Immunology** D. Wier (ed) Blackwell (1973). Plateau  
35        concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12  $\mu$ M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in: **Manual of Clinical Immunology**, 2d Ed. (Rose and Friedman, Eds.) Amer. Soc. For Microbiol., Washington, D.C. (1980).



Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample. The antibodies can also be used in therapeutic compositions for killing bacterial cells  
5 expressing the protein.

### EXAMPLE 13

#### Construction of Strains Which Overexpress or Underexpress Gene Products Required for Proliferation by Promoter Replacement

Strains which overexpress or underexpress gene products required for proliferation may  
10 also be constructed by replacing the promoters which naturally direct transcription of these gene products with promoters which provide the desired level of expression. As described above, such strains are useful in methods for identifying essential genes, in methods for identifying compounds which inhibit cellular proliferation, in methods for identifying the targets of compounds which inhibit proliferation, as well as in methods for identifying genes encoding gene products required  
15 for proliferation. Some embodiments of the present invention contemplate the use of a vector that comprises a regulatable fusion promoter selected from a suite of fusion promoters wherein the promoter suite is useful for modulating both the basal and maximal levels of transcription of a nucleic acid over a wide dynamic range thus allowing the desired level of production of a transcript which corresponds to a nucleic acid described herein. Such promoters are described in U.S. Patent  
20 Application Serial Number 10/032,393, filed December 21, 2001, the disclosure of which is incorporated herein by reference in its entirety.

For example, in some embodiments, the natural promoter may be replaced using techniques which employ homologous recombination to exchange a promoter present on the chromosome of the cell with the desired promoter. In such methodology, a nucleic acid comprising a promoter  
25 replacement cassette is introduced into the cell. As illustrated in Figure 1A, the promoter replacement cassette comprises a 5' region homologous to the sequence which is 5' of the natural promoter in the chromosome, the promoter which is to replace the chromosomal promoter and a 3' region which is homologous to sequences 3' of the natural promoter in the chromosome. In some embodiments, the promoter replacement cassette may also include a nucleic acid encoding an  
30 identifiable or selectable marker disposed between the 5' region which is homologous to the sequence 5' of the natural promoter and the promoter which is to replace the chromosomal promoter. If desired, the promoter replacement cassette may also contain a transcriptional terminator 3' of the gene encoding an identifiable or selectable marker, as illustrated in Figure 1B. As illustrated in Figure 1A and 1B, homologous recombination is allowed to occur between the  
35 chromosomal region containing the natural promoter and the promoter replacement cassette. Cells in which the promoter replacement cassette has integrated into the chromosome are identified or selected. To confirm that homologous recombination has occurred, the chromosomal structure of the cells may be verified by Southern analysis or PCR.

In some embodiments, the promoter replacement cassette may be introduced into the cell as a linear nucleic acid, such a PCR product or a restriction fragment. Alternatively, the promoter replacement may be introduced into the cell on a plasmid. Figures 1A and 1B illustrates the replacement of a chromosomal promoter with a desired promoter through homologous recombination.

In some embodiments, the cell into which the promoter replacement cassette is introduced may carry mutations which enhance its ability to be transformed with linear DNA or which enhance the frequency of homologous recombination. For example, if the cell is an *Escherichia coli* cell it may have a mutation in the gene encoding Exonuclease V of the RecBCD recombination complex. If the cell is an *Escherichia coli* cell it may have a mutation that activates the RecET recombinase of the  $\lambda$  prophage and/or a mutation that enhances recombination through the RecF pathway. For example, the *Escherichia coli* cells may be RecB or RecC mutants carrying an sbcA or sbcB mutation. Alternatively, the *Escherichia coli* cells may be recD mutants. In other embodiments the *Escherichia coli* cells may express the  $\lambda$  Red recombination genes. For example, *Escherichia coli* cells suitable for use in techniques employing homologous recombination have been described in Datsenko, K.A. and Wanner, B.L., PNAS 97:6640-6645 (2000); Murphy, K.C., J. Bact 180: 2053-2071 (1998); Zhang, Y., et al., Nature Genetics 20: 123-128 (1998); and Muirers, J.P.P. et al., Genes & Development 14: 1971-1982 (2000). It will be appreciated that cells carrying mutations in similar genes may be constructed in organisms other than *Escherichia coli*.

In some embodiments, the methods described in U.S. Patent Application Serial Number 09/948,993, may be used to place the gene required for proliferation under the control of a regulatable promoter.

If the organism in which promoter replacement is to be performed is diploid, strains in which genes encoding gene products required for proliferation are under the control of a desired promoter may be constructed by inactivating one chromosomal copy of a gene encoding a gene product required for proliferation. For example, the gene may be inactivated by insertion of or replacement by a nucleotide sequence encoding a selectable or detectable gene product, such as a polypeptide which provides resistance to a drug or which allows growth under certain culture conditions. The other chromosomal copy of the gene encoding a gene product required for proliferation is placed under the control of a regulatable promoter, such as the tetracycline regulatable promoter similar to that described in Gari et al., (1997) *Yeast* 13:837-848 and Nagahashi et al., (1997) *Mol. Gen. Genet.* 255:372-375, by homologous recombination. The resultant strains may be used to identify genes which encode gene products required for proliferation and in the methods of the present invention.

The method may also be applied to haploid organisms by modifying the single allele of the gene via recombination of the allele with a promoter replacement fragment comprising a nucleotide sequence encoding a heterologous promoter, such that the expression of the gene is conditionally regulated by the heterologous promoter. By repeating this process for a preferred subset of genes in

a haploid pathogenic organism, or its entire genome, a collection or a complete set of conditional mutant strains can be obtained.

It will be appreciated that the means to achieve conditional expression are not restricted to the promoters discussed above and can be performed with other conditional promoters. Such conditional promoter may, for example, be regulated by a repressor which repress transcription from the promoter under particular condition or by a transactivator which increases transcription from the promoter, such as, when in the presence of an inducer.

Although not mandatory, performing the gene disruption first enables heterozygous strains to be constructed and separately collected as a heterozygote strain collection during the process of drug target validation. Heterozygous strains for a given gene express approximately half the normal diploid level of a particular gene product. Consequently, these strains provide constructions having a diminished level of the encoded gene product, and they may be used in the methods described herein. However, it is clear to those skilled in the art that the order of allele modification followed in this embodiment of the invention is not critical, and that it is feasible to perform these steps in a different order such that the conditional-expressing allele is constructed first and the disruption of the remaining wild type gene allele be performed subsequently. However, where the promoter replacement step is carried out first, it is preferable to delete sequences homologous to those employed in the gene disruption step.

Alternatively, conditional expression could be achieved by means other than the reliance of conditional promoters. For example, conditional expression could be achieved by the replacement of the wild type allele in haploid or heterozygous strains with temperature sensitive alleles derived *in vitro*, and their phenotype would then be analyzed at the nonpermissive temperature. In a related approach, in heterozygous strains, insertion of a ubiquitination signal into the remaining wild type allele to destabilize the gene product during activation conditions can be adopted to examine phenotypic effects resulting from gene inactivation.

In another alternative, a constitutive promoter regulated by an excisable transactivator can be used. The promoter is placed upstream to a target gene to repress expression to the basal level characteristic of the promoter. For example, if the strains are fungal organisms, a heterologous promoter containing *lexA* operator elements may be used in combination with a fusion protein composed of the *lexA* DNA binding domain and any transcriptional activator domain (e.g. GAL4, HAP4, VP16) to provide constitutive expression of a target gene. Counterselection mediated by 5-FOA can be used to select those cells which have excised the gene encoding the fusion protein. This procedure enables an examination of the phenotype associated with repression of the target gene to the basal level of expression provided by the *lexA* heterologous promoter in the absence of a functional transcription activator. The strains generated by this approach may be used in the present invention.

Alternatively, conditional expression of a target gene can be achieved without the use of a transactivator containing a DNA binding, transcriptional activator domain. A cassette could be

assembled to contain a heterologous constitutive promoter downstream of, for example, the URA3 selectable marker, which is flanked with a direct repeat containing homologous sequences to the 5' portion of the target gene. Additional homologous sequences upstream of the target, when added to this cassette would facilitate homologous recombination and replacement of the native promoter with e above-described heterologous promoter cassette immediately upstream of the start codon of the target gene or open reading frame. Conditional expression is achieved by selecting strains, by using 5-FOA containing media, which have excised the heterologous constitutive promoter and URA3 marker (and consequently lack those regulatory sequences upstream of the target gene required for expression of the gene) and examining the growth of the resulting strain *versus* a wild type strain grown under identical conditions.

#### EXAMPLE 14

##### Promoter Replacement to Generate Cells Capable of Overexpressing or Underexpressing a Gene Encoding a Gene Product Required for Proliferation

A target for promoter replacement is selected. A promoter replacement cassette is obtained by inserting a nucleic acid comprising the *rrnBT1T2* transcriptional terminator followed by the *lac* promoter into pACYC184 such that the *rrnB* terminator and *lac* promoter are positioned 3' of the CAT gene. The promoter replacement cassette (CAT-*rrnBT1T2*-*plac*) is amplified by PCR. The PCR product is used as the template for another round of PCR using primers with 60-80 bp of homology to a target promoter (i.e. a promoter which directs expression of a gene encoding a gene product required for proliferation) and 20 bp of homology to the CAT/*rrnBT1T2*/*plac* template as described above. The region of homology is chosen such that upon homologous recombination, the CAT/*rrnBT1T2*/*plac* cassette replaces the promoter of the target gene but leaves its Shine-Delgarno motif untouched.

The promoter replacement cassette is transformed into competent JC8679. JC8679 is available from the E. coli genetics stock center. JC8679 allows recombination of short linear DNAs and also contains a *lacY* mutation which allows titratable regulation of the *lac* promoter. The transformed cells are plated onto LB/chloramphenicol plates containing various levels of IPTG to assure that the correct level of expression is achieved to allow survival. The correct integration of the promoter replacement cassette is confirmed by colony PCR. If desired, proper regulation of the target gene by the inserted promoter may be confirmed by testing the integrants for growth defects when inducer is absent or present at levels lower than that at which the original colonies were obtained. The inability to grow in the absence of inducer (IPTG) or in the presence of lower levels of the inducer than were used to obtain the clones confirms that the target gene is properly regulated by the inserted promoter. It will be appreciated that although the *lac* promoter and the strain JC8679 are used as examples, the method may be performed using any suitable regulatable promoter and organism or strain to generate cells which are capable of overexpressing or underexpressing a gene encoding a gene product required for proliferation. Examples of promoters that are useful for the regulating the expression of gene products in Gram-positive organisms over a

wide dynamic range are described in U.S. Patent Application Serial Number 10/032,393, filed December 21, 2001.

The following example describes one method for promoter replacement in a prokaryotic cell. It will be appreciated that promoter replacement can be used in a variety of organisms as previously indicated.

#### EXAMPLE 15

##### Operator Insertion to Generate Cells Capable of Overexpressing or Underexpressing a Gene Encoding a Gene Product Required for Proliferation

An oligonucleotide comprising a lac operator flanked on each side by 40 nucleotides homologous to the target promoter is designed. The target promoter is the promoter which drives expression of a gene encoding a gene product required for proliferation, such as the *yabB yabC ftsL ftsI murE* genes in an operon. The sequence of the oligonucleotide (SEQ ID NO. 78582) and locations of the regions homologous to the promoter are illustrated in Figure 6. The sequence of the promoter is also shown with the locations of the -10 and -35 regions indicated (SEQ ID NO. 78583). The single stranded oligonucleotide is transformed into a bacterium expressing the  $\lambda$  Beta and Gam proteins. The cells in the transformation mixture are diluted and plated on medium containing IPTG. Colonies in which the lac operator has integrated into the target promoter are identified by colony PCR. If desired, proper regulation of the target promoter by the inserted operator is confirmed by growing the identified colonies in medium containing or lacking IPTG. The colonies proliferate on medium containing IPTG but fail to grow on medium lacking IPTG, thereby confirming that the target promoter is properly regulated by the inserted operator. It will be appreciated that the preceding method may be performed with any target promoter and any operator to generate cells which overexpress or underexpress a gene encoding a gene product required for proliferation.

#### EXAMPLE 16

##### Screening Chemical Libraries

###### A. Protein-Based Assays

Having isolated and expressed bacterial proteins shown to be required for bacterial proliferation, the present invention further contemplates the use of these expressed target proteins in assays to screen libraries of compounds for potential drug candidates. The generation of chemical libraries is well known in the art. For example, combinatorial chemistry can be used to generate a library of compounds to be screened in the assays described herein. A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis by combining a number of chemical "building block" reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining amino acids in every possible combination to yield peptides of a given length. Millions of chemical compounds theoretically can be synthesized through such combinatorial mixings of chemical building blocks. For example, one commentator observed that the systematic, combinatorial mixing of 100 interchangeable

chemical building blocks results in the theoretical synthesis of 100 million tetrameric compounds or 10 billion pentameric compounds. (Gallop et al., "Applications of Combinatorial Technologies to Drug Discovery, Background and Peptide Combinatorial Libraries," *Journal of Medicinal Chemistry*, Vol. 37, No. 9, 1233-1250 (1994). Other chemical libraries known to those in the art may also be used, including natural product libraries.

Once generated, combinatorial libraries can be screened for compounds that possess desirable biological properties. For example, compounds which may be useful as drugs or to develop drugs would likely have the ability to bind to the target protein identified, expressed and purified as discussed above. Further, if the identified target protein is an enzyme, candidate compounds would likely interfere with the enzymatic properties of the target protein. For example, the enzymatic function of a target protein may be to serve as a protease, nuclease, phosphatase, dehydrogenase, transporter protein, transcriptional enzyme, and any other type of enzyme known or unknown. Thus, the present invention contemplates using the protein products described above to screen combinatorial chemical libraries.

In one example, the target protein is a serine protease and the substrate of the enzyme is known. The present example is directed towards the analysis of libraries of compounds to identify compounds that function as inhibitors of the target enzyme. First, a library of small molecules is generated using methods of combinatorial library formation well known in the art. U.S. Patent Nos. 5,463,564 and 5,574, 656, to Agrafiotis, et al., entitled "System and Method of Automatically Generating Chemical Compounds with Desired Properties," are two such teachings. Then the library compounds are screened to identify those compounds that possess desired structural and functional properties. U.S. Patent No. 5,684,711, also discusses a method for screening libraries.

To illustrate the screening process, the target polypeptide and chemical compounds of the library are combined with one another and permitted to interact with one another. A labeled substrate is added to the incubation. The label on the substrate is such that a detectable signal is emitted from the products of the substrate molecules that result from the activity of the target polypeptide. The emission of this signal permits one to measure the effect of the combinatorial library compounds on the enzymatic activity of target enzymes by comparing it to the signal emitted in the absence of combinatorial library compounds. The characteristics of each library compound are encoded so that compounds demonstrating activity against the enzyme can be analyzed and features common to the various compounds identified can be isolated and combined into future iterations of libraries.

Once a library of compounds is screened, subsequent libraries are generated using those chemical building blocks that possess the features shown in the first round of screen to have activity against the target enzyme. Using this method, subsequent iterations of candidate compounds will possess more and more of those structural and functional features required to inhibit the function of the target enzyme, until a group of enzyme inhibitors with high specificity for the enzyme can be found. These compounds can then be further tested for their safety and efficacy as antibiotics for use in mammals.

It will be readily appreciated that this particular screening methodology is exemplary only. Other methods are well known to those skilled in the art. For example, a wide variety of screening techniques are known for a large number of naturally-occurring targets when the biochemical function of the target protein is known. For example, some techniques involve the generation and use of small peptides to probe and analyze target proteins both biochemically and genetically in order to identify and develop drug leads. Such techniques include the methods described in PCT publications No. WO9935494, WO9819162, WO9954728. Other techniques utilize natural product libraries or libraries of larger molecules such as proteins.

It will be appreciated that the above protein-based assays may be performed with any of the proliferation-required polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* (including the polypeptides of SEQ ID NOs.: 42398-78581) or portions thereof. In addition, the above protein-based assays may be performed with homologous polypeptides or portions thereof.

#### B. Cell-Based Assays

Current cell-based assays used to identify or to characterize compounds for drug discovery and development frequently depend on detecting the ability of a test compound to modulate the activity of a target molecule located within a cell or located on the surface of a cell. An advantage of cell-based assays is that they allow the effect of a compound on a target molecule's activity to be detected within the physiologically relevant environment of the cell as opposed to an *in vitro* environment. Most often such target molecules are proteins such as enzymes, receptors and the like. However, target molecules may also include other molecules such as DNAs, lipids, carbohydrates and RNAs including messenger RNAs, ribosomal RNAs, tRNAs, regulatory RNAs and the like. A number of highly sensitive cell-based assay methods are available to those of skill in the art to detect binding and interaction of test compounds with specific target molecules. However, these methods are generally not highly effective when the test compound binds to or otherwise interacts with its target molecule with moderate or low affinity. In addition, the target molecule may not be

readily accessible to a test compound in solution, such as when the target molecule is located inside the cell or within a cellular compartment. Thus, current cell-based assay methods are limited in that they are not effective in identifying or characterizing compounds that interact with their targets with moderate to low affinity or compounds that interact with targets that are not readily accessible.

5       The cell-based assay methods of the present invention have substantial advantages over current cell-based assays. These advantages derive from the use of sensitized cells in which the level or activity of at least one proliferation-required gene product (the target molecule) has been specifically reduced to the point where the presence or absence of its function becomes a rate-determining step for cellular proliferation. Bacterial, fungal, plant, or animal cells can all be used  
10       with the present method. Such sensitized cells become much more sensitive to compounds that are active against the affected target molecule. Thus, cell-based assays of the present invention are capable of detecting compounds exhibiting low or moderate potency against the target molecule of interest because such compounds are substantially more potent on sensitized cells than on non-sensitized cells. The effect may be such that a test compound may be two to several times more  
15       potent, at least 10 times more potent, at least 20 times more potent, at least 50 times more potent, at least 100 times more potent, at least 1000 times more potent, or even more than 1000 times more potent when tested on the sensitized cells as compared to the non-sensitized cells. The proliferation-required nucleic acids or polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*,  
20       *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria*  
25       *monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus*  
30       *pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis*, or portions thereof, may be employed in any of the cell-based assays described herein. Similarly, homologous coding nucleic acids, homologous antisense nucleic acids, or homologous polypeptides or portions of the homologous nucleic acids or homologous polypeptides, may be employed in any of the cell-based assays described herein.

35       Due in part to the increased appearance of antibiotic resistance in pathogenic microorganisms and to the significant side-effects associated with some currently used antibiotics, novel antibiotics acting at new targets are highly sought after in the art. Yet, another limitation in the current art related to cell-based assays is the problem of repeatedly identifying hits against the



same kinds of target molecules in the same limited set of biological pathways. This may occur when compounds acting at such new targets are discarded, ignored or fail to be detected because compounds acting at the "old" targets are encountered more frequently and are more potent than compounds acting at the new targets. As a result, the majority of antibiotics in use currently  
5 interact with a relatively small number of target molecules within an even more limited set of biological pathways.

The use of sensitized cells of the current invention provides a solution to the above problem in two ways. First, desired compounds acting at a target of interest, whether a new target or a previously known but poorly exploited target, can now be detected above the "noise" of compounds  
10 acting at the "old" targets due to the specific and substantial increase in potency of such desired compounds when tested on the sensitized cells of the current invention. Second, the methods used to sensitize cells to compounds acting at a target of interest may also sensitize these cells to compounds acting at other target molecules within the same biological pathway. For example, expression of an antisense molecule to a gene encoding a ribosomal protein is expected to sensitize  
15 the cell to compounds acting at that ribosomal protein and may also sensitize the cells to compounds acting at any of the ribosomal components (proteins or rRNA) or even to compounds acting at any target which is part of the protein synthesis pathway. Thus an important advantage of the present invention is the ability to reveal new targets and pathways that were previously not readily accessible to drug discovery methods.

Sensitized cells of the present invention are prepared by reducing the activity or level of a target molecule. The target molecule may be a gene product, such as an RNA or polypeptide produced from the proliferation-required nucleic acids from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*,  
25 *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* (including a gene product produced from the  
35 nucleic acids of SEQ ID NOs.: 6214-42397, such as the polypeptides of SEQ ID NOs.: 42398-78581) or from homologous nucleic acids. For example, the target molecule may be one of the polypeptides of SEQ ID NOs. 42398-78581 or a homologous polypeptide. Alternatively, the target

may be a gene product such as an RNA or polypeptide which is produced from a sequence within the same operon as the proliferation-required nucleic acids *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or from homologous nucleic acids. In addition, the target may be an RNA or polypeptide in the same biological pathway as the proliferation-required nucleic acids from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or from homologous nucleic acids. Such biological pathways include, but are not limited to, enzymatic, biochemical and metabolic pathways as well as pathways involved in the production of cellular structures such as the cell wall.

Current methods employed in the arts of medicinal and combinatorial chemistries are able to make use of structure-activity relationship information derived from testing compounds in various biological assays including direct binding assays and cell-based assays. Occasionally compounds are directly identified in such assays that are sufficiently potent to be developed as drugs. More often, initial hit compounds exhibit moderate or low potency. Once a hit compound is identified with low or moderate potency, directed libraries of compounds are synthesized and tested

in order to identify more potent leads. Generally these directed libraries are combinatorial chemical libraries consisting of compounds with structures related to the hit compound but containing systematic variations including additions, subtractions and substitutions of various structural features. When tested for activity against the target molecule, structural features are identified that either alone or in combination with other features enhance or reduce activity. This information is used to design subsequent directed libraries containing compounds with enhanced activity against the target molecule. After one or several iterations of this process, compounds with substantially increased activity against the target molecule are identified and may be further developed as drugs. This process is facilitated by use of the sensitized cells of the present invention since compounds acting at the selected targets exhibit increased potency in such cell-based assays, thus; more compounds can now be characterized providing more useful information than would be obtained otherwise.

Thus, it is now possible using cell-based assays of the present invention to identify or characterize compounds that previously would not have been readily identified or characterized including compounds that act at targets that previously were not readily exploited using cell-based assays. The process of evolving potent drug leads from initial hit compounds is also substantially improved by the cell-based assays of the present invention because, for the same number of test compounds, more structure-function relationship information is likely to be revealed.

The method of sensitizing a cell entails selecting a suitable gene or operon. A suitable gene or operon is one whose transcription and/or expression is required for the proliferation of the cell to be sensitized. The next step is to introduce into the cells to be sensitized, an antisense RNA capable of hybridizing to the suitable gene or operon or to the RNA encoded by the suitable gene or operon. Introduction of the antisense RNA can be in the form of a vector in which antisense RNA is produced under the control of an inducible promoter. The amount of antisense RNA produced is modulated by varying an inducer concentration to which the cell is exposed and thereby varying the activity of the promoter driving transcription of the antisense RNA. Thus, cells are sensitized by exposing them to an inducer concentration that results in a sub-lethal level of antisense RNA expression. The requisite amount of inducer may be derived empiracally by one of skill in the art.

In one embodiment of the cell-based assays, antisense nucleic acids complementary to the identified *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria*

*meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* nucleotide sequences or portions thereof (including antisense nucleic acids comprising a nucleotide sequence complementary to one of SEQ ID NOs.: 6214-42397, and the antisense nucleic acids of SEQ ID NOs.: 1-6213 or antisense nucleic acids comprising a nucleotide sequence complementary to portions of the foregoing nucleic acids thereof), antisense nucleic complementary to homologous coding nucleic acids or portions thereof or homologous antisense nucleic acids are used to inhibit the production of a proliferation-required protein. Vectors producing antisense RNA complementary to identified genes required for proliferation, or portions thereof, are used to limit the concentration of a proliferation-required protein without severely inhibiting growth. The proliferation-required protein may be one of the proteins of SEQ ID NOs.: 42398-78581 or a homologous polypeptide. To achieve that goal, a growth inhibition dose curve of inducer is calculated by plotting various doses of inducer against the corresponding growth inhibition caused by the antisense expression. From this curve, the concentration of inducer needed to achieve various percentages of antisense induced growth inhibition, from 1 to 100% can be determined.

In some embodiments of the present invention, promoter replacement methods, such as those describe above and in U.S. Patent Application Serial Number 09/948,993, are used to express the proliferation-inhibiting nucleic acid. In other embodiments, the methods for the production of stabilized RNA in Gram-negative organisms, as described in U.S. Provisional Patent Application Serial Number 60/343,512, are used for the production of proliferation-inhibiting transcripts corresponding to the nucleic acid sequences described herein. Briefly, the stabilized antisense RNA may comprise an antisense RNA which was identified as inhibiting proliferation as described above which has been engineered to contain at least one stem loop flanking each end of the antisense nucleic acid. In some embodiments, the at least one stem-loop structure formed at the 5' end of the stabilized antisense nucleic acid comprises a flush, double stranded 5' end. In some embodiments, one or more of the stem loops comprises a rho independent terminator. In additional embodiments, the stabilized antisense RNA lacks a ribosome binding site. In further embodiments, the stabilized RNA lacks sites which are cleaved by one or more RNAses, such as RNase E or RNase III. In some embodiments, the stabilized antisense RNA may be transcribed in a cell which the activity of at least one enzyme involved in RNA degradation has been reduced. For example, the activity of an enzyme such as RNase E, RNase II, RNase III, polynucleotide phosphorylase, and poly(A) polymerase, RNA helicase, enolase or an enzyme having similar functions may be reduced in the cell.

A variety of different regulatable promoters may be used to produce the antisense nucleic acid. Transcription from the regulatable promoters may be modulated by controlling the activity of a transcription factor repressor which acts at the regulatable promoter. For example, if transcription

is modulated by affecting the activity of a repressor, the choice of inducer to be used depends on the repressor/operator responsible for regulating transcription of the antisense nucleic acid. If the regulatable promoter comprises a T5 promoter fused to a *xyIO* (xylose operator; e.g. derived from *Staphylococcus xylosis* (Schnappinger, D. et al., FEMS Microbiol. Let. 129: 126214-423978 (1995)), then transcription of the antisense nucleic acid may be regulated by a xylose repressor. The xylose repressor may be provided by ectopic expression within an *S. aureus* cell of an exogenous xylose repressor gene, e.g. derived from *S. xylosis* DNA. In such cases transcription of antisense RNA from the promoter is inducible by adding xylose to the medium and the promoter is thus "xylose inducible." Similarly, IPTG inducible promoters may be used. For example, the highest concentration of the inducer that does not reduce the growth rate significantly can be estimated from the curve. Cellular proliferation can be monitored by growth medium turbidity via OD measurements. In another example, the concentration of inducer that reduces growth by 25% can be predicted from the curve. In still another example, a concentration of inducer that reduces growth by 50% can be calculated. Additional parameters such as colony forming units (cfu) can be used to measure cellular viability. Some embodiments of the present invention contemplate the use of a vector that comprises a regulatable fusion promoter selected from a suite of fusion promoters wherein the promoter suite is useful for modulating both the basal and maximal levels of transcription of a nucleic acid over a wide dynamic range thus allowing the desired level of production of a transcript which corresponds to a nucleic acid described herein. Such promoters are described in U.S. Patent Application Serial Number 10/032,393, filed December 21, 2001, the disclosure of which is incorporated herein by reference in its entirety.

Cells to be assayed are exposed to the above-determined concentrations of inducer. The presence of the inducer at this sub-lethal concentration reduces the amount of the proliferation required gene product to a sub-optimal amount in the cell that will still support growth. Cells grown in the presence of this concentration of inducer are therefore specifically more sensitive to inhibitors of the proliferation-required protein or RNA of interest or to inhibitors of proteins or RNAs in the same biological pathway as the proliferation-required protein or RNA of interest but not to inhibitors of unrelated proteins or RNAs.

Cells pretreated with sub-inhibitory concentrations of inducer and thus containing a reduced amount of proliferation-required target gene product are then used to screen for compounds that reduce cell growth. The sub-lethal concentration of inducer may be any concentration consistent with the intended use of the assay to identify candidate compounds to which the cells are more sensitive. For example, the sub-lethal concentration of the inducer may be such that growth inhibition is at least about 5%, at least about 8%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60% at least about 75%, or more. Cells which are pre-sensitized using the preceding method are more sensitive to inhibitors of the target protein because these cells contain less target protein to inhibit than do wild-type cells.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids comprising a nucleotide sequence complementary to any of the proliferation-required nucleic acids from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*,  
5 *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*,  
10 *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*,  
15 *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*,  
20 *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or homologous polypeptides.

In another embodiment of the cell-based assays of the present invention, the level or activity of a proliferation required gene product is reduced using a mutation, such as a temperature sensitive mutation, in the gene encoding a gene product required for proliferation and an antisense  
35 nucleic acid comprising a nucleotide sequence complementary to the gene encoding the gene product required for proliferation or a portion thereof. Growing the cells at an intermediate temperature between the permissive and restrictive temperatures of the temperature sensitive mutant where the mutation is in a proliferation-required gene produces cells with reduced activity

of the proliferation-required gene product. The antisense RNA complementary to the proliferation-required sequence further reduces the activity of the proliferation required gene product. Drugs that may not have been found using either the temperature sensitive mutation or the antisense nucleic acid alone may be identified by determining whether cells in which transcription of the antisense nucleic acid has been induced and which are grown at a temperature between the permissive temperature and the restrictive temperature are substantially more sensitive to a test compound than cells in which expression of the antisense nucleic acid has not been induced and which are grown at a permissive temperature. Also drugs found previously from either the antisense nucleic acid alone or the temperature sensitive mutation alone may have a different sensitivity profile when used in cells combining the two approaches, and that sensitivity profile may indicate a more specific action of the drug in inhibiting one or more activities of the gene product.

Temperature sensitive mutations may be located at different sites within the gene and correspond to different domains of the protein. For example, the *dnaB* gene of *Escherichia coli* encodes the replication fork DNA helicase. DnaB has several domains, including domains for oligomerization, ATP hydrolysis, DNA binding, interaction with primase, interaction with DnaC, and interaction with DnaA [(Biswas, E.E. and Biswas, S.B. 1999. Mechanism and DnaB helicase of *Escherichia coli*: structural domains involved in ATP hydrolysis, DNA binding, and oligomerization. *Biochem.* 38:10919-10928; Hiasa, H. and Marians, K.J. 1999. Initiation of bidirectional replication at the chromosomal origin is directed by the interaction between helicase and primase. *J. Biol. Chem.* 274:27244-27248; San Martin, C., Radermacher, M., Wolpensinger, B., Engel, A., Miles, C.S., Dixon, N.E., and Carazo, J.M. 1998. Three-dimensional reconstructions from cryoelectron microscopy images reveal an intimate complex between helicase DnaB and its loading partner DnaC. *Structure* 6:501-9; Sutton, M.D., Carr, K.M., Vicente, M., and Kaguni, J.M. 1998. *Escherichia coli* DnaA protein. The N-terminal domain and loading of DnaB helicase at the *E. coli* chromosomal origin. *J. Biol. Chem.* 273:34255-62.). Temperature sensitive mutations in different domains of DnaB confer different phenotypes at the restrictive temperature, which include either an abrupt stop or slow stop in DNA replication with or without DNA breakdown (Wechsler, J.A. and Gross, J.D. 1971. *Escherichia coli* mutants temperature-sensitive for DNA synthesis. *Mol. Gen. Genetics* 113:273-284, and termination of growth or cell death. Combining the use of temperature sensitive mutations in the *dnaB* gene that cause cell death at the restrictive temperature with an antisense to the *dnaB* gene could lead to the discovery of very specific and effective inhibitors of one or a subset of activities exhibited by DnaB.

It will be appreciated that the above method may be performed with any mutation which reduces but does not eliminate the activity or level of the gene product which is required for proliferation.

It will be appreciated that the above cell-based assays may be performed using mutations in, such as temperature sensitive mutations, and antisense nucleic acids comprising a nucleotide sequence complementary to any of the genes encoding proliferation-required gene products from

from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*,  
5 *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria*  
10 *meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or portions thereof (including the nucleic acids of SEQ ID NOs.: 6214-42397), mutations in and antisense  
15 nucleic acids complementary to homologous coding nucleic acids or portions thereof or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*,  
20 *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*,  
25 *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio*  
30 *cholerae* or *Yersinia pestis* (including the polypeptides of SEQ ID NOs.: 42398-78581), or homologous polypeptides may be reduced.

When screening for antimicrobial agents against a gene product required for proliferation, growth inhibition of cells containing a limiting amount of that proliferation-required gene product can be assayed. Growth inhibition can be measured by directly comparing the amount of growth,  
35 measured by the optical density of the growth medium, between an experimental sample and a control sample. Alternative methods for assaying cell proliferation include measuring the signal from a reporter construct, various enzymatic activity assays, and other methods well known in the art.



It will be appreciated that the above method may be performed in solid phase, liquid phase or a combination of the two. For example, cells grown on nutrient agar containing the inducer of the antisense construct may be exposed to compounds spotted onto the agar surface. If desired, the cells may be grown on agar containing varying concentrations of the inducer. A compound's effect may be judged from the diameter of the resulting killing zone, the area around the compound application point in which cells do not grow. Multiple compounds may be transferred to agar plates and simultaneously tested using automated and semi-automated equipment including but not restricted to multi-channel pipettes (for example the Beckman Multimek) and multi-channel spotters (for example the Genomic Solutions Flexys). In this way multiple plates and thousands to millions of compounds may be tested per day.

The compounds may also be tested entirely in liquid phase using microtiter plates as described below. Liquid phase screening may be performed in microtiter plates containing 96, 384, 1536 or more wells per microtiter plate to screen multiple plates and thousands to millions of compounds per day. Automated and semi-automated equipment may be used for addition of reagents (for example cells and compounds) and determination of cell density.

#### EXAMPLE 17

##### Cell-Based Assay Using Antisense Complementary to Genes Encoding Ribosomal Proteins

The effectiveness of the above cell-based assay was validated using constructs transcribing antisense RNA to the proliferation required *E. coli* genes *rplL*, *rplJ*, and *rplW* encoding ribosomal proteins L7/L12, L10 and L23 respectively. These proteins are essential components of the protein synthesis apparatus of the cell and as such are required for proliferation. These constructs were used to test the effect of antisense transcription on cell sensitivity to antibiotics known to bind to the ribosome and thereby inhibit protein synthesis. Constructs transcribing antisense RNA to several other genes (*elaD*, *visC*, *yohH*, and *atpE/B*), the products of which are not involved in protein synthesis were used for comparison.

First, pLex5BA (Krause et al., J. Mol. Biol. 274: 365 (1997)), vectors containing antisense constructs to either *rplW* or to *elaD* were introduced into separate *E. coli* cell populations. Vector introduction is a technique well known to those of ordinary skill in the art. The vectors of this example contain IPTG inducible promoters that drive the transcription of the antisense RNA in the presence of the inducer. However, those skilled in the art will appreciate that other inducible promoters may also be used. Suitable vectors are also well known in the art. For example, a number of promoters useful for nucleic acid transcription (including the nucleic acids described herein) in *Enterococcus faecalis*, *Staphylococcus aureus* as well as other Gram positive organisms are described in U.S. Patent Application Serial Number 10/032,393, filed December 21, 2001. Antisense clones to genes encoding different ribosomal proteins or to genes encoding proteins that are not involved in protein synthesis were utilized to test the effect of antisense transcription on cell sensitivity to the antibiotics known to bind to ribosomal proteins and inhibit protein synthesis. Antisense nucleic acids comprising a nucleotide sequence complementary to the *elaD*, *atpB* & *atpE*,

*visC* and *yohH* genes are referred to as AS-*elaD*, AS-*atpB/E*, AS-*visC*, AS-*yohH* respectively. These genes are not known to be involved in protein synthesis. Antisense nucleic acids to the *rplL*, *rplL&rplJ* and *rplW* genes are referred to as AS-*rplL*, AS-*rplL/J*, and AS-*rplW* respectively. These genes encode ribosomal proteins L7/L12 (*rplL*) L10 (*rplJ*) and L23 (*rplW*). Vectors containing these antisense nucleic acids were introduced into separate *E. coli* cell populations.

The cell populations containing vectors producing AS-*elaD* or AS-*rplW* were exposed to a range of IPTG concentrations in liquid medium to obtain the growth inhibitory dose curve for each clone (Figure 7). First, seed cultures were grown to a particular turbidity measured by the optical density (OD) of the growth solution. The OD of the solution is directly related to the number of bacterial cells contained therein. Subsequently, sixteen 200 µl liquid medium cultures were grown in a 96 well microtiter plate at 37° C with a range of IPTG concentrations in duplicate two-fold serial dilutions from 1600 µM to 12.5 µM (final concentration). Additionally, control cells were grown in duplicate without IPTG. These cultures were started from an inoculum of equal amounts of cells derived from the same initial seed culture of a clone of interest. The cells were grown for up to 15 hours and the extent of growth was determined by measuring the optical density of the cultures at 600 nm. When the control culture reached mid-log phase the percent growth (relative to the control culture) for each of the IPTG containing cultures was plotted against the log concentrations of IPTG to produce a growth inhibitory dose response curve for the IPTG. The concentration of IPTG that inhibits cell growth to 50% (IC<sub>50</sub>) as compared to the 0 mM IPTG control (0% growth inhibition) was then calculated from the curve. Under these conditions, an amount of antisense RNA was produced that reduced the expression levels of *rplW* or *elaD* to a degree such that growth of cells containing their respective antisense vectors was inhibited by 50%.

Alternative methods of measuring growth are also contemplated. Examples of these methods include measurements of proteins, the expression of which is engineered into the cells being tested and can readily be measured. Examples of such proteins include luciferase and various enzymes.

Cells were pretreated with the selected concentration of IPTG and then used to test the sensitivity of cell populations to tetracycline, erythromycin and other known protein synthesis inhibitors. Figure 7 is an IPTG dose response curve in *E. coli* transformed with an IPTG-inducible plasmid containing either an antisense clone to the *E. coli* *rplW* gene (AS-*rplW*) which encodes ribosomal protein L23 which is required for protein synthesis and essential for cell proliferation, or an antisense clone to the *elaD* (AS-*elaD*) gene which is not known to be involved in protein synthesis.

An example of a tetracycline dose response curve is shown in Figures 8A and 13B for the *rplW* and *elaD* genes, respectively. Cells were grown to log phase and then diluted into medium alone or medium containing IPTG at concentrations which give 20% and 50% growth inhibition as determined by IPTG dose response curves. After 2.5 hours, the cells were diluted to a final OD<sub>600</sub> of 0.002 into 96 well plates containing (1) +/- IPTG at the same concentrations used for the 2.5 hour

pre-incubation; and (2) serial two-fold dilutions of tetracycline such that the final concentrations of tetracycline range from 1 µg/ml to 15.6 ng/ml and 0 µg/ml. The 96 well plates were incubated at 37°C and the OD<sub>600</sub> was read by a plate reader every 5 minutes for up to 15 hours. For each IPTG concentration and the no IPTG control, tetracycline dose response curves were determined when the control (absence of tetracycline) reached 0.1 OD<sub>600</sub>.

To compare tetracycline sensitivity with and without IPTG, tetracycline IC<sub>50s</sub> were determined from the dose response curves (Figures 8A-B). Cells transcribing antisense nucleic acids AS-*rplL* or AS-*rplW* to genes encoding ribosomal proteins L7/L12 and L23 respectively showed increased sensitivity to tetracycline (Figure 8A) as compared to cells with reduced levels of the *elaD* gene product (AS-*elaD*) (Figure 8B). Figure 9 shows a summary bar chart in which the ratios of tetracycline IC<sub>50s</sub> determined in the presence of IPTG which gives 50% growth inhibition versus tetracycline IC<sub>50s</sub> determined without IPTG (fold increase in tetracycline sensitivity) were plotted. Cells with reduced levels of either L7/L12 (encoded by genes *rplL*, *rplJ*) or L23 (encoded by the *rplW* gene) showed increased sensitivity to tetracycline (Figure 9). Cells expressing antisense to genes not known to be involved in protein synthesis (AS-*atpB/E*, AS-*visC*, AS-*elaD*, AS-*yohH*) did not show the same increased sensitivity to tetracycline, validating the specificity of this assay (Figure 9).

In addition to the above, it has been observed in initial experiments that clones transcribing antisense RNA to genes involved in protein synthesis (including genes encoding ribosomal proteins L7/L12 & L10, L7/L12 alone, L22, and L18, as well as genes encoding rRNA and Elongation Factor G) have increased sensitivity to the macrolide, erythromycin, whereas clones transcribing antisense to the non-protein synthesis genes *elaD*, *atpB/E* and *visC* do not. Furthermore, the clone transcribing antisense to *rplL* and *rplJ* (AS-*rplL/J*) does not show increased sensitivity to nalidixic acid and ofloxacin, antibiotics which do not inhibit protein synthesis.

The results with the ribosomal protein genes *rplL*, *rplJ*, and *rplW* as well as the initial results using various other antisense clones and antibiotics show that limiting the concentration of an antibiotic target makes cells more sensitive to the antimicrobial agents that specifically interact with that protein. The results also show that these cells are sensitized to antimicrobial agents that inhibit the overall function in which the protein target is involved but are not sensitized to antimicrobial agents that inhibit other functions. It will be appreciated that the cell-based assays described above may be implemented using the *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*,

- Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* antisense nucleotide sequences which inhibit the activity of genes required for proliferation described herein (including the antisense nucleic acids of SEQ ID NOs.: 1-6213) or antisense nucleic acids comprising nucleotide sequences which are complementary to the sequences of SEQ ID NOs.: 6214-42397 or portions thereof.
- 10 It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids complementary to any of the proliferation-required nucleic acids from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*,
- 15 *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or portions thereof, antisense nucleic acids
- 20 complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*,
- 25 *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*,
- 30 *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*,
- 35 *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*,

*Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or homologous polypeptides may be reduced.

In some embodiments of the present invention, the methods for the production of stabilized RNA, as described in U.S. Provisional Patent Application Serial Number 60/343,512, can be used  
 5 in the above cell-based assays in Gram-negative organisms to extend the lifetime of transcripts corresponding to the nucleic acids described herein.

The cell-based assay described above may also be used to identify the biological pathway in which a proliferation-required nucleic acid or its gene product lies. In such methods, cells transcribing a sub-lethal level of antisense to a target proliferation-required nucleic acid and control  
 10 cells in which transcription of the antisense has not been induced are contacted with a panel of antibiotics known to act in various pathways. If the antibiotic acts in the pathway in which the target proliferation-required nucleic acid or its gene product lies, cells in which transcription of the antisense has been induced will be more sensitive to the antibiotic than cells in which expression of the antisense has not been induced.

As a control, the results of the assay may be confirmed by contacting a panel of cells  
 15 transcribing antisense nucleic acids to many different proliferation-required genes including the target proliferation-required gene. If the antibiotic is acting specifically, heightened sensitivity to the antibiotic will be observed only in the cells transcribing antisense to a target proliferation-required gene (or cells expressing antisense to other proliferation-required genes in the same  
 20 pathway as the target proliferation-required gene) but will not be observed generally in all cells expressing antisense to proliferation-required genes.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids complementary to any of the proliferation-required nucleic acids from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas*  
 25 *aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter*  
 30 *pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus*  
 35 *mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* (including antisense nucleic acids complementary to SEQ ID NOs: 6214-42397, or the antisense nucleic acids of SEQ ID NOs: 1-6213) or portions thereof, antisense nucleic acids comprising nucleotide sequences complementary to homologous

coding nucleic acids or portions thereof, or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* (including the polypeptides of SEQ ID NOs.: 42398-78581), or homologous polypeptides may be reduced.

In some embodiments of the present invention, the methods for the production of stabilized RNA, as described in U.S. Provisional Patent Application Serial Number 60/343,512, can be used in the above cell-based assays in Gram-negative organisms to extend the lifetime of transcripts corresponding to the nucleic acids described herein.

Similarly, the above method may be used to determine the pathway on which a test compound, such as a test antibiotic acts. A panel of cells, each of which transcribes an antisense to a proliferation-required nucleic acid in a known pathway, is contacted with a compound for which it is desired to determine the pathway on which it acts. The sensitivity of the panel of cells to the test compound is determined in cells in which transcription of the antisense has been induced and in control cells in which expression of the antisense has not been induced. If the test compound acts on the pathway on which an antisense nucleic acid acts, cells in which expression of the antisense has been induced will be more sensitive to the compound than cells in which expression of the antisense has not been induced. In addition, control cells in which expression of antisense to proliferation-required genes in other pathways has been induced will not exhibit heightened sensitivity to the compound. In this way, the pathway on which the test compound acts may be determined.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids comprising nucleotide sequences complementary to any of the proliferation-required nucleic acids from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia*

*pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*,  
5 *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or  
10 *Yersinia pestis* (including antisense nucleic acids complementary to SEQ ID NOs: 6214-42397, such as the antisense nucleic acids of SEQ ID NOs: 1-6213) or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*,  
15 *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*,  
20 *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*,  
25 *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* (including the polypeptides of SEQ ID NOs: 42398-78581) or homologous polypeptides may be reduced.

In some embodiments of the present invention, the methods for the production of stabilized  
30 RNA, as described in U.S. Provisional Patent Application Serial Number 60/343,512, can be used in the above cell-based assays in Gram-negative organisms to extend the lifetime of transcripts corresponding to the nucleic acids described herein.

The Example below provides one method for performing such assays.

#### EXAMPLE 18

35 Identification of the Pathway in which a Proliferation-Required  
Gene Lies or the Pathway on which an Antibiotic Acts

##### A. Preparation of Bacterial Stocks for Assay

To provide a consistent source of cells to screen, frozen stocks of host bacteria containing the desired antisense construct are prepared using standard microbiological techniques. For example, a single clone of the microorganism can be isolated by streaking out a sample of the original stock onto an agar plate containing nutrients for cell growth and an antibiotic for which the antisense construct contains a selectable marker which confers resistance. After overnight growth an isolated colony is picked from the plate with a sterile needle and transferred to an appropriate liquid growth medium containing the antibiotic required for maintenance of the plasmid. The cells are incubated at 30°C to 37°C with vigorous shaking for 4 to 6 hours to yield a culture in exponential growth. Sterile glycerol is added to 15% (volume to volume) and 100µL to 500 µL aliquots are distributed into sterile cryotubes, snap frozen in liquid nitrogen, and stored at -80°C for future assays.

**B. Growth of Bacteria for Use in the Assay**

A day prior to an assay, a stock vial is removed from the freezer, rapidly thawed (37°C water bath) and a loop of culture is streaked out on an agar plate containing nutrients for cell growth and an antibiotic to which the selectable marker of the antisense construct confers resistance. After overnight growth at 37°C, ten randomly chosen, isolated colonies are transferred from the plate (sterile inoculum loop) to a sterile tube containing 5 mL of LB medium containing the antibiotic to which the antisense vector confers resistance. After vigorous mixing to form a homogeneous cell suspension, the optical density of the suspension is measured at 600 nm (OD<sub>600</sub>) and if necessary an aliquot of the suspension is diluted into a second tube of 5 mL, sterile, LB medium plus antibiotic to achieve an OD<sub>600</sub> ≤ 0.02 absorbance units. The culture is then incubated at 37° C for 1-2 hrs with shaking until the OD<sub>600</sub> reaches OD 0.2 – 0.3. At this point the cells are ready to be used in the assay.



C. Selection of Media to be Used in Assay

Two-fold dilution series of the inducer are generated in culture media containing the appropriate antibiotic for maintenance of the antisense construct. Several media are tested side by side and three to four wells are used to evaluate the effects of the inducer at each concentration in each media. For example, LB broth, TBD broth and Muller-Hinton media may be tested with the inducer xylose at the following concentrations, 5 mM, 10 mM, 20 mM, 40 mM, 80 mM, 120 mM and 160 mM. Equal volumes of test media-inducer and cells are added to the wells of a 384 well microtiter plate and mixed. The cells are prepared as described above and diluted 1:100 in the appropriate media containing the test antibiotic immediately prior to addition to the microtiter plate wells. For a control, cells are also added to several wells of each media that do not contain inducer, for example 0 mM xylose. Cell growth is monitored continuously by incubation at 37°C in a microtiter plate reader monitoring the OD<sub>600</sub> of the wells over an 18-hour period. The percent inhibition of growth produced by each concentration of inducer is calculated by comparing the rates of logarithmic growth against that exhibited by cells growing in medium without inducer. The medium yielding greatest sensitivity to inducer is selected for use in the assays described below.

D. Measurement of Test Antibiotic Sensitivity in the Absence of Antisense Construct Induction

Two-fold dilution series of antibiotics of known mechanism of action are generated in the culture medium selected for further assay development that has been supplemented with the antibiotic used to maintain the construct. A panel of test antibiotics known to act on different pathways is tested side by side with three to four wells being used to evaluate the effect of a test antibiotic on cell growth at each concentration. Equal volumes of test antibiotic and cells are added to the wells of a 384 well microtiter plate and mixed. Cells are prepared as described above using the medium selected for assay development supplemented with the antibiotic required to maintain the antisense construct and are diluted 1:100 in identical medium immediately prior to addition to the microtiter plate wells. For a control, cells are also added to several wells that lack antibiotic, but contain the solvent used to dissolve the antibiotics. Cell growth is monitored continuously by incubation at 37°C in a microtiter plate reader monitoring the OD<sub>600</sub> of the wells over an 18-hour period. The percent inhibition of growth produced by each concentration of antibiotic is calculated by comparing the rates of logarithmic growth against that exhibited by cells growing in medium without antibiotic. A plot of percent inhibition against log[antibiotic concentration] allows extrapolation of an IC<sub>50</sub> value for each antibiotic.

E. Measurement of Test Antibiotic Sensitivity in the Presence of Antisense Construct Inducer

The culture medium selected for use in the assay is supplemented with inducer at concentrations shown to inhibit cell growth by 50% and 80% as described above, as well as the antibiotic used to maintain the construct. Two-fold dilution series of the panel of test antibiotics used above are generated in each of these media. Several antibiotics are tested side by side in each medium with three to four wells being used to evaluate the effects of an antibiotic on cell growth at each concentration. Equal volumes of test antibiotic and cells are added to the wells of a 384 well

microtiter plate and mixed. Cells are prepared as described above using the medium selected for use in the assay supplemented with the antibiotic required to maintain the antisense construct. The cells are diluted 1:100 into two 50 mL aliquots of identical medium containing concentrations of inducer that have been shown to inhibit cell growth by 50% and 80 % respectively and incubated at 37°C with shaking for 2.5 hours. Immediately prior to addition to the microtiter plate wells, the cultures are adjusted to an appropriate OD<sub>600</sub> (typically 0.002) by dilution into warm (37°C) sterile medium supplemented with identical concentrations of the inducer and antibiotic used to maintain the antisense construct. For a control, cells are also added to several wells that contain solvent used to dissolve test antibiotics but which contain no antibiotic. Cell growth is monitored continuously by incubation at 37°C in a microtiter plate reader monitoring the OD<sub>600</sub> of the wells over an 18-hour period. The percent inhibition of growth produced by each concentration of antibiotic is calculated by comparing the rates of logarithmic growth against that exhibited by cells growing in medium without antibiotic. A plot of percent inhibition against log[antibiotic concentration] allows extrapolation of an IC<sub>50</sub> value for each antibiotic.

**F. Determining the Specificity of the Test Antibiotics**

A comparison of the IC<sub>50</sub>s generated by antibiotics of known mechanism of action under antisense induced and non-induced conditions allows the pathway in which a proliferation-required nucleic acid lies to be identified. If cells expressing an antisense nucleic acid comprising a nucleotide sequence complementary to a proliferation-required gene are selectively sensitive to an antibiotic acting via a particular pathway, then the gene against which the antisense acts is involved in the pathway on which the antibiotic acts.

**G. Identification of Pathway in which a Test Antibiotic Acts**

As discussed above, the cell-based assay may also be used to determine the pathway against which a test antibiotic acts. In such an analysis, the pathways against which each member of a panel of antisense nucleic acids acts are identified as described above. A panel of cells, each containing an inducible vector which transcribes an antisense nucleic acid comprising a nucleotide sequence complementary to a gene in a known proliferation-required pathway, is contacted with a test antibiotic for which it is desired to determine the pathway on which it acts under inducing and non-inducing conditions. If heightened sensitivity is observed in induced cells transcribing antisense complementary to a gene in a particular pathway but not in induced cells transcribing antisense nucleic acids comprising nucleotide sequences complementary to genes in other pathways, then the test antibiotic acts against the pathway for which heightened sensitivity was observed.

One skilled in the art will appreciate that further optimization of the assay conditions, such as the concentration of inducer used to induce antisense transcription and/or the growth conditions used for the assay (for example incubation temperature and medium components) may further increase the selectivity and/or magnitude of the antibiotic sensitization exhibited.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids comprising nucleotide sequences complementary to any of the proliferation-required nucleic acids from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*,  
5 *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*,  
10 *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*,  
15 *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* (including antisense nucleic acids comprising nucleotide sequences complementary to SEQ ID NOs: 6214-42397, such as the antisense nucleic acids of SEQ ID NOs: 1-6213) or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids. In this way, the level or activity of a  
20 target, such as any of the proliferation-required polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*,  
25 *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*,  
30 *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* (including the polypeptides of SEQ ID NOs: 42398-78581), or homologous polypeptides may be reduced.

35 In some embodiments of the present invention, the methods for the production of stabilized RNA, as described in U.S. Provisional Patent Application Serial Number 60/343,512, can be used in the above cell-based assays in Gram-negative organisms to extend the lifetime of transcripts corresponding to the nucleic acids described herein.

The following example confirms the effectiveness of the methods described above.

### EXAMPLE 19

#### Identification of the Biological Pathway in which a Proliferation-Required Gene Lies

The effectiveness of the above assays was validated using proliferation-required genes from  
5 *E. coli* which were identified using procedures similar to those described above. Antibiotics of various chemical classes and modes of action were purchased from Sigma Chemicals (St. Louis, MO). Stock solutions were prepared by dissolving each antibiotic in an appropriate aqueous solution based on information provided by the manufacturer. The final working solution of each antibiotic contained no more than 0.2% (w/v) of any organic solvent. To determine their potency  
10 against a bacterial strain engineered for transcription of an antisense comprising a nucleotide sequence complementary to a proliferation-required 50S ribosomal protein, each antibiotic was serially diluted two- or three- fold in growth medium supplemented with the appropriate antibiotic for maintenance of the antisense construct. At least ten dilutions were prepared for each antibiotic. 25  $\mu$ L aliquots of each dilution were transferred to discrete wells of a 384-well microplate (the  
15 assay plate) using a multi-channel pipette. Quadruplicate wells were used for each dilution of an antibiotic under each treatment condition (plus and minus inducer). Each assay plate contained twenty wells for cell growth controls (growth medium replacing antibiotic), ten wells for each treatment (plus and minus inducer, in this example IPTG). Assay plates were usually divided into the two treatments: half the plate containing induced cells and an appropriate concentrations of  
20 inducer (in this example IPTG) to maintain the state of induction, the other half containing non-induced cells in the absence of IPTG.

Cells for the assay were prepared as follows. Bacterial cells containing a construct, from which transcription of antisense nucleic acid comprising a nucleotide sequence complementary to *rplL* and *rplJ* (AS-*rplL/J*), which encode proliferation-required 50S ribosomal subunit proteins, is  
25 inducible in the presence of IPTG, were grown into exponential growth ( $OD_{600}$  0.2 to 0.3) and then diluted 1:100 into fresh medium containing either 400  $\mu$ M or 0  $\mu$ M inducer (IPTG). These cultures were incubated at 37° C for 2.5 hr. After a 2.5 hr incubation, induced and non-induced cells were respectively diluted into an assay medium at a final  $OD_{600}$  value of 0.0004. The medium contained an appropriate concentration of the antibiotic for the maintenance of the antisense construct. In  
30 addition, the medium used to dilute induced cells was supplemented with 800  $\mu$ M IPTG so that addition to the assay plate would result in a final IPTG concentration of 400  $\mu$ M. Induced and non-induced cell suspensions were dispensed (25  $\mu$ L/well) into the appropriate wells of the assay plate as discussed previously. The plate was then loaded into a plate reader, incubated at constant temperature, and cell growth was monitored in each well by the measurement of light scattering at  
35 595 nm. Growth was monitored every 5 minutes until the cell culture attained a stationary growth phase. For each concentration of antibiotic, a percentage inhibition of growth was calculated at the time point corresponding to mid-exponential growth for the associated control wells (no antibiotic, plus or minus IPTG). For each antibiotic

or minus IPTG), a plot of percent

inhibition versus log of antibiotic concentration was generated and the  $IC_{50}$  determined. A comparison of the  $IC_{50}$  for each antibiotic in the presence and absence of IPTG revealed whether induction of the antisense construct sensitized the cell to the mechanism of action exhibited by the antibiotic. Cells which exhibited a statistically significant decrease in the  $IC_{50}$  value in the presence  
5 of inducer were considered to have an increased sensitivity to the test antibiotic.

The results are provided in the table below, which lists the classes and names of the antibiotics used in the analysis, the targets of the antibiotics, the  $IC_{50}$  in the absence of IPTG, the  $IC_{50}$  in the presence of IPTG, the concentration units for the  $IC_{50}$ s, the fold increase in  $IC_{50}$  in the presence of IPTG, and whether increased sensitivity was observed in the presence of IPTG.

TABLE V  
Effect of Expression of Antisense RNA to *rplL* and *rplJ* on Antibiotic Sensitivity

ANTIBIOTIC CLASS /Names	TARGET	IC <sub>50</sub> (-IPTG)	IC <sub>50</sub> (+IPTG)	Conc. Unit	Fold Increase in Sensitivity	Sensitivity Increased?
<b>PROTEIN SYNTHESIS INHIBITOR</b>						
<b>AMINOGLYCOSIDES</b>						
Gentamicin	30S ribosome function	2715	19.19	ng/ml	141	Yes
Streptomycin	30S ribosome function	11280	161	ng/ml	70	Yes
Spectinomycin	30S ribosome function	18050	<156	ng/ml		Yes
Tobramycin	30S ribosome function	3594	70.58	ng/ml	51	Yes
<b>MACROLIDES</b>						
Erythromycin	50S ribosome function	7467	187	ng/ml	40	Yes
<b>AROMATIC POLYKETIDES</b>						
Tetracycline	30S ribosome function	199.7	1.83	ng/ml	109	Yes
Minocycline	30S ribosome function	668.4	3.897	ng/ml	172	Yes
Doxycycline	30S ribosome function	413.1	27.81	ng/ml	15	Yes
<b>OTHER PROTEIN SYNTHESIS INHIBITORS</b>						
Fusidic acid	Elongation Factor G function	59990	641	ng/ml	94	Yes
Chloramphenicol	30S ribosome function	465.4	1.516	ng/ml	307	Yes
Lincomycin	50S ribosome function	47150	324.2	ng/ml	145	Yes
<b>OTHER ANTIBIOTIC MECHANISMS</b>						
<b>B-LACTAMS</b>						
Cefoxitin	Cell wall biosynthesis	2782	2484	ng/ml	1	No
Cefotaxime	Cell wall biosynthesis	24.3	24.16	ng/ml	1	No
<b>DNA SYNTHESIS INHIBITORS</b>						
Nalidixic acid	DNA Gyrase activity	6973	6025	ng/ml	1	No
Ofloxacin	DNA Gyrase activity	49.61	45.89	ng/ml	1	No
<b>OTHER</b>						
Bacitracin	Cell membrane function	4077	4677	mg/ml	1	No
Trimethoprim	Dihydrofolate Reductase activity	128.9	181.97	ng/ml	1	No
Vancomycin	Cell wall biosynthesis	145400	72550	ng/ml	2	No

The above results demonstrate that induction of an antisense RNA complementary to genes encoding 50S ribosomal subunit proteins results in a selective and highly significant sensitization of cells to antibiotics that inhibit ribosomal function and protein synthesis. The above results further demonstrate that induction of an antisense to an essential gene sensitizes a cell or microorganism to compounds that interfere with that gene product's biological role. This sensitization is restricted to compounds that interfere with pathways associated with the targeted gene and its product.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids complementary to any of the proliferation-required nucleic acids from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* (including antisense nucleic acids complementary to SEQ ID NOs. 6214-42397, such as the antisense nucleic acids of SEQ ID NOs. 1-6213) or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*,

*Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*,  
5 *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* (including the polypeptides of SEQ ID NOs.: 42398-78581), or homologous polypeptides may be reduced.

In some embodiments of the present invention, the methods for the production of stabilized RNA, as described in U.S. Provisional Patent Application Serial Number 60/343,512,  
10 can be used in the above cell-based assays in Gram-negative organisms to extend the lifetime of transcripts corresponding to the nucleic acids described herein.

Example 20 below describes an analysis performed in *Staphylococcus aureus*.

#### EXAMPLE 20

##### Identification of the Biological Pathway in which a Gene Required for 15 Proliferation of *Staphylococcus aureus* Lies

Antibiotics of various chemical classes and modes of action were purchased from chemical suppliers, for example Sigma Chemicals (St. Louis, MO). Stock solutions were prepared by dissolving each antibiotic in an appropriate aqueous solution based on information provided by the manufacturer. The final working solution of each antibiotic contained no more  
20 than 0.2% (w/v) of any organic solvent.

To determine its potency against a bacterial strain containing an antisense nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence encoding the Beta subunit of DNA gyrase (which is required for proliferation) under the control of a xylose inducible promoter, each antibiotic was serially diluted two- or three- fold in growth medium supplemented with the appropriate antibiotic for maintenance of the antisense construct. At  
25 least ten dilutions were prepared for each antibiotic.

Aliquots (25  $\mu$ L) of each dilution were transferred to discrete wells of a 384-well microplate (the assay plate) using a multi-channel pipette. Quadruplicate wells were used for each dilution of an antibiotic under each treatment condition (plus and minus inducer). Each  
30 assay plate contained twenty wells for cell growth controls (growth medium, no antibiotic), ten wells for each treatment (plus and minus inducer, xylose, in this example). Half the assay plate contained induced cells (in this example *Staphylococcus aureus* cells) and appropriate concentrations of inducer ( xylose, in this example) to maintain the state of induction while the other half of the assay plate contained non-induced cells maintained in the absence of inducer.

35



#### Preparation of Bacterial Cells

Cells of a bacterial clone containing a construct in which transcription of antisense comprising a nucleotide sequence complementary to the sequence encoding the Beta subunit of DNA gyrase under the control of the xylose inducible promoter (S1M10000001F08) were  
5 grown into exponential growth ( $OD_{600}$  0.2 to 0.3) and then diluted 1:100 into fresh medium containing either 12 mM or 0 mM inducer (xylose). These cultures were incubated at 37° C for 2.5 hr. The presence of inducer (xylose) in the medium initiates and maintains production of antisense RNA from the antisense construct. After a 2.5 hr incubation, induced and non-induced cells were respectively diluted into an assay medium containing an appropriate  
10 concentration of the antibiotic for the maintenance of the antisense construct. In addition, medium used to dilute induced cells was supplemented with 24 mM xylose so that addition to the assay plate would result in a final xylose concentration of 12 mM. The cells were diluted to a final  $OD_{600}$  value of 0.0004.

Induced and non-induced cell suspensions were dispensed (25  $\mu$ l/well) into the  
15 appropriate wells of the assay plate as discussed previously. The plate was then loaded into a plate reader and incubated at constant temperature while cell growth was monitored in each well by the measurement of light scattering at 595 nm. Growth was monitored every 5 minutes until the cell culture attained a stationary growth phase. For each concentration of antibiotic, a percentage inhibition of growth was calculated at the time point corresponding to mid-exponential growth for the associated control wells (no antibiotic, plus or minus xylose). For  
20 each antibiotic and condition (plus or minus xylose), plots of percent inhibition versus Log of antibiotic concentration were generated and  $IC_{50}$ s determined.

A comparison of each antibiotic's  $IC_{50}$  in the presence and absence of inducer (xylose, in this example) reveals whether induction of the antisense construct sensitized the cell to the  
25 antibiotic's mechanism of action. If the antibiotic acts against the  $\beta$  subunit of DNA gyrase, the  $IC_{50}$  of induced cells will be significantly lower than the  $IC_{50}$  of uninduced cells.

Figure 10 lists the antibiotics tested, their targets, and their fold increase in potency between induced cells and uninduced cells. As illustrated in Figure 10, the potency of cefotaxime, cefoxitin, fusidic acid, lincomycin, tobramycin, trimethoprim and vancomycin,  
30 each of which act on targets other than the  $\beta$  subunit of gyrase, was not significantly different in induced cells as compared to uninduced cells. However, the potency of novobiocin, which is known to act against the Beta subunit of DNA gyrase, was significantly different between induced cells and uninduced cells.

Thus, induction of an antisense nucleic acid comprising a nucleotide sequence  
35 complementary to the sequence encoding the  $\beta$  subunit of gyrase results in a selective and

significant sensitization of *Staphylococcus aureus* cells to an antibiotic which inhibits the activity of this protein. Furthermore, the results demonstrate that induction of an antisense construct to an essential gene sensitizes a cell or microorganism to compounds that interfere with that gene product's biological role. This sensitization is apparently restricted to compounds that interfere with the targeted gene and its product.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids complementary to any of the proliferation-required nucleic acids from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* (including antisense nucleic acids complementary to SEQ ID NOs.: 6214-42397, such as the antisense nucleic acids of SEQ ID NOs. 1-6213), or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus*

*mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or homologous polypeptides may be reduced.

5 In some embodiments of the present invention, the methods for the production of stabilized RNA, as described in U.S. Provisional Patent Application Serial Number 60/343,512, can be used in the above cell-based assays in Gram-negative organisms to extend the lifetime of transcripts corresponding to the nucleic acids described herein.

10 Assays utilizing antisense constructs to essential genes or portions thereof can be used to identify compounds that interfere with the activity of those gene products. Such assays could be used to identify drug leads, for example antibiotics.

Panels of cells transcribing different antisense nucleic acids can be used to characterize the point of intervention of a compound affecting an essential biochemical pathway including antibiotics with no known mechanism of action.

15 Assays utilizing antisense constructs to essential genes can be used to identify compounds that specifically interfere with the activity of multiple targets in a pathway. Such constructs can be used to simultaneously screen a sample against multiple targets in one pathway in one reaction (Combinatorial HTS).

20 Furthermore, as discussed above, panels of antisense construct-containing cells may be used to characterize the point of intervention of any compound affecting an essential biological pathway including antibiotics with no known mechanism of action.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids complementary to any of the proliferation-required nucleic acids from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*,  
 25 *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*,  
 30 *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*,  
 35 *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*,

*Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* (including antisense nucleic acids comprising nucleotide sequences complementary to SEQ ID NOs.: 6214-42397, such as the antisense nucleic acids of SEQ ID NOs. 1-6213), or portions thereof, antisense nucleic acids  
 5 complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*,  
 15 *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*,  
 20 *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or homologous polypeptides may be reduced.

In some embodiments of the present invention, the methods for the production of stabilized RNA, as described in U.S. Provisional Patent Application Serial Number 60/343,512, can be used in the above cell-based assays in Gram-negative organisms to extend the lifetime of  
 25 transcripts corresponding to the nucleic acids described herein.

Another embodiment of the present invention is a method for determining the pathway against which a test antibiotic compound is active, in which the activity of target proteins or nucleic acids involved in proliferation-required pathways is reduced by contacting cells with a sub-lethal concentration of a known antibiotic which acts against the target protein or nucleic  
 30 acid. In one embodiment, the target protein or nucleic acid corresponds to a proliferation-required nucleic acid identified using the methods described above, such as the polypeptides of SEQ ID NOs.: 42398-78581, or homologous polypeptides. The method is similar to those described above for determining which pathway a test antibiotic acts against, except that rather than reducing the activity or level of a proliferation-required gene product using a sub-lethal  
 35 level of antisense to a proliferation-required nucleic acid, the sensitized cell is generated by

reducing the activity or level of the proliferation-required gene product using a sub-lethal level of a known antibiotic which acts against the proliferation required gene product. Heightened sensitivity determines the pathway on which the test compound is active.

Interactions between drugs which affect the same biological pathway have been described in the literature. For example, Mecillinam (Amdinocillin) binds to and inactivates the penicillin binding protein 2 (PBP2, product of the *mrda* in *E. coli*). This antibiotic interacts with other antibiotics that inhibit PBP2 as well as antibiotics that inhibit other penicillin binding proteins such as PBP3 [(Gutmann, L., Vincent, S., Billot-Klein, D., Acar, J.F., Mrena, E., and Williamson, R. (1986) Involvement of penicillin-binding protein 2 with other penicillin-binding proteins in lysis of *Escherichia coli* by some beta-lactam antibiotics alone and in synergistic lytic effect of amdinocillin (mecillinam). *Antimicrobial Agents & Chemotherapy*, 30:906-912). Interactions between drugs could, therefore, involve two drugs that inhibit the same target protein or nucleic acid or inhibit different proteins or nucleic acids in the same pathway [(Fukuoka, T., Domon, H., Kakuta, M., Ishii, C., Hirasawa, A., Utsui, Y., Ohya, S., and Yasuda, H. (1997) Combination effect between panipenem and vancomycin on highly methicillin-resistant *Staphylococcus aureus*. *Japan. J. Antibio.* 50:411-419; Smith, C.E., Foleno, B.E., Barrett, J.F., and Frosc, M.B. (1997) Assessment of the synergistic interactions of levofloxacin and ampicillin against *Enterococcus faecium* by the checkerboard agar dilution and time-kill methods. *Diagnos. Microbiol. Infect. Disease* 27:85-92; den Hollander, J.G., Horrevorts, A.M., van Goor, M.L., Verbrugh, H.A., and Mouton, J.W. (1997) Synergism between tobramycin and ceftazidime against a resistant *Pseudomonas aeruginosa* strain, tested in an in vitro pharmacokinetic model. *Antimicrobial Agents & Chemotherapy*. 41:95-110).

Two drugs may interact even though they inhibit different targets. For example, the proton pump inhibitor, Omeprazole, and the antibiotic, Amoxycillin, two synergistic compounds acting together, can cure *Helicobacter pylori* infection [( Gabryelewicz, A., Laszewicz, W., Dzieniszewski, J., Ciok, J., Marlicz, K., Bielecki, D., Popiela, T., Legutko, J., Knapik, Z., Poniewierka, E. (1997) Multicenter evaluation of dual-therapy (omeprazol and amoxycillin) for *Helicobacter pylori*-associated duodenal and gastric ulcer (two years of the observation). *J. Physiol. Pharmacol.* 48 Suppl 4:93-105).

The growth inhibition from the sub-lethal concentration of the known antibiotic may be at least about 5%, at least about 8%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, or at least about 75%, or more.

Alternatively, the sub-lethal concentration of the known antibiotic may be determined by measuring the activity of the target proliferation-required gene product rather than by measuring growth inhibition.

Cells are contacted with a combination of each member of a panel of known antibiotics at a sub-lethal level and varying concentrations of the test antibiotic. As a control, the cells are contacted with varying concentrations of the test antibiotic alone. The  $IC_{50}$  of the test antibiotic in the presence and absence of the known antibiotic is determined. If the  $IC_{50}$ s in the presence and absence of the known drug are substantially similar, then the test drug and the known drug act on different pathways. If the  $IC_{50}$ s are substantially different, then the test drug and the known drug act on the same pathway.

It will be appreciated that the above cell-based assays may be performed using a sub-lethal concentration of a known antibiotic which acts against the product of any of the proliferation-required nucleic acids from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* (including the products of SEQ ID NOs: 6214-42397, or portions thereof, or the products of homologous coding nucleic acids or portions thereof). In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria*

*meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* (including the polypeptides of SEQ ID NOs.: 42398-78581), or homologous polypeptides may be reduced.

Another embodiment of the present invention is a method for identifying a candidate compound for use as an antibiotic in which the activity of target proteins or nucleic acids involved in proliferation-required pathways is reduced by contacting cells with a sub-lethal concentration of a known antibiotic which acts against the target protein or nucleic acid. In one embodiment, the target protein or nucleic acid is a target protein or nucleic acid corresponding to a proliferation-required nucleic acid identified using the methods described above. The method is similar to those described previously herein for identifying candidate compounds for use as antibiotics except that rather than reducing the activity or level of a proliferation-required gene product using a sub-lethal level of antisense to a proliferation-required nucleic acid, the activity or level of the proliferation-required gene product is reduced using a sub-lethal level of a known antibiotic which acts against the proliferation required gene product.

The growth inhibition from the sub-lethal concentration of the known antibiotic may be at least about 5%, at least about 8%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, or at least about 75%, or more.

Alternatively, the sub-lethal concentration of the known antibiotic may be determined by measuring the activity of the target proliferation-required gene product rather than by measuring growth inhibition.

In order to characterize test compounds of interest, cells are contacted with a panel of known antibiotics at a sub-lethal level and one or more concentrations of the test compound. As a control, the cells are contacted with the same concentrations of the test compound alone. The  $IC_{50}$  of the test compound in the presence and absence of the known antibiotic is determined. If the  $IC_{50}$  of the test compound is substantially different in the presence and absence of the known drug then the test compound is a good candidate for use as an antibiotic. As discussed above, once a candidate compound is identified using the above methods its structure may be optimized using standard techniques such as combinatorial chemistry.

Representative known antibiotics which may be used in each of the above methods are provided in Table VI below. However, it will be appreciated that other antibiotics may also be used.

**TABLE VI**  
**Antibiotics and Their Targets**

ANTIBIOTIC	INHIBITS/TARGET	RESISTANT MUTANTS
<b>Inhibitors of Transcription</b>		
Rifamycin, Rifampicin	Inhibits initiation of transcription/ $\beta$ -subunit RNA polymerase, <i>rpoB</i>	<i>rpoB</i> , <i>crp</i> , <i>cyaA</i>
Rifabutin Rifaximin	Accelerates transcription chain termination/ $\beta$ -subunit RNA polymerase	<i>rpoB</i>
Streptolydigin	an acyclic ansamycin, inhibits RNA polymerase	<i>rpoB</i>
Streptovaricin	Intercalates between 2 successive G-C pairs, <i>rpoB</i> , inhibits RNA synthesis	<i>pldA</i>
Actinomycin D+EDTA		
<b>Inhibitors of Nucleic Acid Metabolism</b>		
Quinolones,	$\alpha$ subunit gyrase and/or topoisomerase IV, <i>gyrA</i>	<i>gyrA</i> or <i>B</i> , <i>icd</i> , <i>sloB</i>
Nalidixic acid Oxolinic acid		
Fluoroquinolones	$\alpha$ subunit gyrase, <i>gyrA</i> and/or topoisomerase IV (probable target in Staph)	<i>gyrA</i>
Ciprofloxacin, Norfloxacin		<i>norA</i> (efflux in Staph)
Coumerins	Inhibits ATPase activity of $\beta$ -subunit gyrase, <i>gyrB</i>	<i>hipQ</i>
Novobiocin		<i>gyrB</i> , <i>cysB</i> , <i>cysE</i> , <i>nov</i> , <i>ompA</i>
Coumermycin	Inhibits ATPase activity of $\beta$ -subunit gyrase, <i>gyrB</i>	<i>gyrB</i> , <i>hisW</i>
Albicidin	DNA synthesis	<i>tsx</i> (nucleoside channel)
Metronidazole	Causes single-strand breaks in DNA	<i>nar</i>
<b>Inhibitors of Metabolic Pathways</b>		
Sulfonamides,	blocks synthesis of dihydrofolate, dihydro-pterolate synthesis, <i>folP</i>	<i>folP</i> , <i>gpt</i> , <i>pabA</i> , <i>pabB</i> , <i>pabC</i>
Sulfanilamide		
Trimethoprim, Showdomycin	Inhibits dihydrofolate reductase, <i>folA</i>	<i>folA</i> , <i>thyA</i>
	Nucleoside analogue capable of alkylating sulfhydryl groups, inhibitor of thymidylate synthetase	<i>nupC</i> , <i>pnp</i>
Thiolactomycin	type II fatty acid synthase inhibitor	<i>emrB</i>
		<i>fadB</i> , <i>emrB</i> due to gene dosage
Psicofuranine	Adenosine glycoside antibiotic, target is GMP synthetase	<i>guaA</i> , <i>B</i>
Triclosan	Inhibits fatty acid synthesis	<i>fabI</i> ( <i>envM</i> )
Diazaborines Isoniazid, Ethionamide	heterocyclic, contain boron, inhibit fatty acid synthesis, enoyl-ACP reductase, <i>fabI</i>	<i>fabI</i> ( <i>envM</i> )



**Inhibitors of Translation**

Phenylpropanoids Chloramphenicol,	Binds to ribosomal peptidyl transfer center preventing peptide translocation/ binds to S6, L3, L6, L14, L16, L25, L26, L27, but preferentially to L16	<i>rrn, cmlA, marA, ompF, ompR</i>
Tetracyclines, type II polyketides Minocycline Doxycycline	Binding to 30S ribosomal subunit, "A" site on 30S subunit, blocks peptide elongation, strongest binding to S7	<i>clmA (cmr), mar, ompF</i>
Macrolides (type I polyketides) Erythromycin, Clarithromycin,	Binding to 50 S ribosomal subunit, 23S rRNA, blocks peptide translocation, L15, L4, L12	<i>rrn, rplC, rplD, rplV, mac</i>
Spiramycin etc		
Aminoglycosides Streptomycin,  Neomycin  Spectinomycin Kanamycin  Kasugamycin  Gentamicin, Amikacin Paromycin	Irreversible binding to 30S ribosomal subunit, prevents translation or causes mistranslation of mRNA/16S rRNA	<i>rpsL, strC,M, ubiF atpA-E, ecjB, hemAC,D,E,G, topA, rpsC,D,E, rrn, spcB atpA-atpE, cpxA, ecjB, hemA,B,L, topA ksgA,B,C,D, rplB,K, rpsI,N,M,R rplF, ubiF cpxA rpsL</i>
Lincosamides Lincomycin, Clindamycin	Binding to 50 S ribosomal subunit, blocks peptide translocation	<i>linB, rplN,O, rpsG</i>
Streptogramins Virginiamycin, Pristinamycin	2 components, Streptogramins A&B, bind to the 50S ribosomal subunit blocking peptide translocation and peptide bond formation	
Synergid: quinupristin /dalbapristin		
Fusidanes Fusidic Acid	Inhibition of elongation factor G (EF-G) prevents peptide translocation	<i>fusA</i>
Kirromycin (Mocimycin)	Inhibition of elongation factor TU (EF-Tu), prevents peptide bond formation	<i>tufA,B</i>
Pulvomycin Thiopeptin	Binds to and inhibits EF-TU Sulfur-containing antibiotic, inhibits protein synthesis, EF-G	<i>rplE</i>
Tiamulin Negamycin	Inhibits protein synthesis Inhibits termination process of protein synthesis	<i>rplC, rplD prfB</i>
Oxazolidinones Linezolid Isoniazid	23S rRNA	
Nitrofurantoin	Inhibits protein synthesis,	<i>pdx nfnA,B</i>

	nitroreductases convert nitrofurantoin to highly reactive electrophilic intermediates which attack bacterial ribosomal proteins non-specifically	
Pseudomonic Acids Mupirocin (Bactroban)	Inhibition of isoleucyl tRNA synthetase-used for Staph, topical cream, nasal spray	<i>ileS</i>
Indolmycin Viomycin	Inhibits tryptophanyl-tRNA synthetase	<i>trpS</i> <i>rrmA</i> (23S rRNA methyltransferase; mutant has slow growth rate, slow chain elongation rate, and viomycin resistance)
Thiopeptides Thiostrepton Micrococcin	Binds to L11-23S RNA complex Inhibits GTP hydrolysis by EF-G Stimulates GTP hydrolysis by EF-G	
<b>Inhibitors of Cell Walls/Membranes</b>		
$\beta$ -lactams Penicillin, Ampicillin Methicillin,	Inhibition of one or more cell wall transpeptidases, endopeptidases, and glycosidases (PBPs), of the 12 PBPs only 2 are essential: <i>mrdA</i> (PBP2) and <i>ftsI</i> ( <i>pbpB</i> , PBP3)	<i>ampC</i> , <i>ampD</i> , <i>ampE</i> , <i>envZ</i> , <i>galU</i> , <i>hipA</i> , <i>hipQ</i> , <i>ompC</i> , <i>ompF</i> , <i>ompR</i> , <i>ptsI</i> , <i>rfa</i> , <i>tolD</i> , <i>tolE</i>
Cephalosporins, Mecillinam (amdinocillin)	Binds to and inactivates PBP2 ( <i>mrdA</i> ) Inactivates PBP3 ( <i>ftsI</i> )	<i>tonB</i> <i>alaS</i> , <i>argS</i> , <i>crp</i> , <i>cyaA</i> , <i>envB</i> , <i>mrdA</i> , <i>B</i> , <i>mreB</i> , <i>C</i> , <i>D</i>
Aztreonam (Furazlocillin) Bacilysin, Tetaine	Dipeptide, inhib glucosamine synthase	<i>dppA</i>
Glycopeptides Vancomycin, Polypeptides Bacitracin	Inhib $G^+$ cell wall syn, binds to terminal D-ala-D-ala of pentapeptide, Prevents dephosphorylation and regeneration of lipid carrier	<i>rfa</i>
Cyclic lipopeptide Daptomycin,	Disrupts multiple aspects of membrane function, including peptidoglycan synthesis, lipoteichoic acid synthesis, and the bacterial membrane potential	
Cyclic polypeptides Polymixin,	Surfactant action disrupts cell membrane lipids, binds lipid A moiety of LPS	<i>pmrA</i>
Fosfomycin,	Analogue of P-enolpyruvate, inhibits 1 <sup>st</sup> step in peptidoglycan synthesis - UDP-N-acetylglucosamine	<i>murA</i> , <i>crp</i> , <i>cyaA</i> <i>glpT</i> , <i>hipA</i> , <i>ptsI</i> , <i>uhpT</i>

	enolpyruvyl transferase, <i>murA</i> . Also acts as Immunosuppressant	
Cycloserine	Prevents formation of D-ala dimer, inhibits D-ala ligase, <i>ddlA,B</i>	<i>hipA, cycA</i>
Alafosfalin	phosphonodipeptide, cell wall synthesis inhibitor, potentiator of $\beta$ -lactams	<i>pepA, tpp</i>

**Inhibitors of Protein Processing/Transport**

Globomycin	Inhibits signal peptidase II (cleaves prolipoproteins subsequent to lipid modification, <i>lspA</i> )	<i>lpp, dnaE</i>
------------	-------------------------------------------------------------------------------------------------------	------------------

It will be appreciated that the above cell-based assays may be performed using a sub-lethal concentration of a known antibiotic which acts against the product of any of the proliferation-required nucleic acids from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or portions thereof, or homologous nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria*

meningitidis, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or homologous polypeptides may be reduced.

#### EXAMPLE 21

Strains in which a Gene Encoding a Gene Product Required for Proliferation is Overexpressed are able to Grow at Elevated Antibiotic Concentrations

To confirm that cells which overexpress a gene product required for proliferation are able to grow at elevated antibiotic concentrations, 11 such genes from *Staphylococcus aureus* which are the targets of known antibiotics were operably linked to the xylose inducible promoter XylT5 (described in U.S. Patent Application Serial Number 10/032,393. The genes and the antibiotics which target the products of these genes are listed in Table VII below.

PCR primer pairs were designed for each of the 11 genes encoding a gene product required for proliferation of *Staphylococcus aureus* as shown in Table VII. The upstream primers for each gene included the native ribosomal binding sites (S-D sequences). In addition, restriction sites for appropriate restriction enzymes were designed into the primers to facilitate directional cloning of the genes. PCR reactions were carried out using Pfu DNA polymerase (Stratagene, San Diego) under the following conditions per 50 µl reaction: Pfu polymerase 2U, dNTP 200 µM, primers 400 nM each, *S. aureus* RN450 genomic DNA (template) 5-10 ng. The reaction involved an initial heating at 94°C for 5 min, followed by 25 cycles of 30 sec at 94°C/30 sec at 55°C/5 min at 72°C, and ending with 7 min of extension at 72°C.

The amplified genes were operably linked to the XylT5 promoter as follows. PCR products were cleaned using QIAGEN PCR Cleaning Kits and then were digested with the proper restriction enzymes. The resulting fragments were ligated overnight at 16°C with precut vector DNA containing the XylT5 promoter. Ligation mixtures were ethanol precipitated at -80°C for 20 min in the presence of 0.3 M sodium acetate. The precipitated DNA was spun down at 14,000 rpm for 30 min at 4°C and washed with 1 ml of 70% EtOH. The DNA pellets were air-dried and dissolved in EB or sterile water. To transform *Staphylococcus aureus* cells, the precipitated DNA was mixed with 45 µl of electroporation competent cells and incubated at room temperature for 30 min. The DNA/cell mixtures were electroporated (settings: 2 volts, 25 µF, 200 Ω) in 2 mm cuvettes and mixed with 450 µl B2 medium containing 0.2 µg/ml chloramphenicol. The cells were incubated at 37°C with shaking for 90 min. Transformed cells were plated onto LB agar plates containing chloramphenicol (34 µg/ml) for the selection of plasmids. Several colonies for each cloning reaction were picked and streaked to obtain a pure

culture. Colony PCR reactions using vector-specific primers were performed to verify the size and identity of the inserts.

Gene-walking sequencing was employed to completely sequence the entire insert for several clones of each cloned gene. This was carried out to avoid using a cloned gene whose  
5 DNA sequence was mutated during the PCR process.

To demonstrate that genes encoding gene products required for proliferation can confer resistance to their specific inhibitors upon induction at proper inducer levels, cells of each clone in which the genes were operably linked to the xylose inducible promoter were grown in LB medium with chloramphenicol (34 µg/ml) at a combination of differing antibiotic and inducer  
10 concentrations. This was accomplished by using microtitration plates (96 or 384 wells) which contained antibiotic and inducer at gradient concentrations in a matrix format in 10 times excess quantity (see Figure 11). Media containing inoculated cells (9 volume) was dispensed into the wells containing 1 volume of antibiotic/inducer for a final volume of 50 µl (for 384 well plates) or 200 µl (for 96 well plates). The plates were incubated at 37°C with periodic shaking and  
15 growth of cells was monitored by automatic measurement of optical density at OD600 using a Ultramark reader. A clone over-expressing a particular gene was considered resistant to its specific antibiotic (inhibitor) if significant growth was observed at appropriate inducer concentrations in the presence of a particular concentration of antibiotic but not in the absence of inducer at that concentration of antibiotic.

The results are indicated in Figure 12 and Figure 13. As illustrated in Figure 12, at  
20 appropriate concentrations of inducer cells which overexpress the *defB* gene product were able to grow at elevated concentrations of the antibiotic actinonin, which acts on the *defB* gene product. Similarly, as illustrated in Figure 13, at appropriate concentrations of inducer cells which overexpress the *folA* gene product were able to grow at elevated concentrations of the  
25 antibiotic trimethoprim, which acts on the *folA* gene product.

Thus, elevated expression of a gene product required for proliferation enables cells to grow in the presence of antibiotic concentrations which inhibit or prevent growth of wild type cells.

Table VII - Essential Genes/Proteins and Specific Inhibitors

Gene	Target	Inhibitor	Primers
<i>gyrB</i>	$\beta$ subunit of DNA gyrase or topoisomerase II	Novobiocin	GCCGGATCCTTATAAAGTAACAGAAAGCGATGGTGACTGC (SEQ ID NO.: 78593); CAGGTCGACCGCCTTAGAAGTCTAAGTTTGCATAAACTG (SEQ ID NO.: 78594)
<i>murA</i>	UDP-N-acetylglucosamine enolpyruvyl transferase	Fosfomycin	CCTGGATCCTTCTAAGTGGAGGATTACG (SEQ ID NO.: 78595); CAGGTCGACGAATTAATCGTTAATACGTT (SEQ ID NO.: 78596)
<i>fabI</i>	Enoyl-acyl carrier protein reductase	Triclosan	GCCGGATCCATAAGGAGTTATCTTACATG (SEQ ID NO.: 78597); CGCGTCGACTTATTTAATTCGTGGAATC (SEQ ID NO.: 78598)
<i>rpoB</i>	RNA polymerase $\beta$ subunit	Rifampicin	GCTGGATCCTGAGGGTGAATCTGTTGGC (SEQ ID NO.: 78599); CTGCTCGAGTGCCTATTAATCAGTAACCTT (SEQ ID NO.: 78600)
<i>fusA</i>	Elongation factor G	Fusidic acid	GCTGGATCCCTGGAAAGGAGAAAAATACATGGCTAGAG (SEQ ID NO.: 78601); CCGGTCGACGGCTAGCTAGTCAAAACAAGTTATATTTCAC (SEQ ID NO.: 78602)
<i>folA</i>	Dihydrofolate reductase	Trimethoprim	GCTGGATCCAGAAAGAGGAGGATAATTATG (SEQ ID NO.: 78603); CCGGTCGACTTTTCCCTTATTTTAC (SEQ ID NO.: 78604)
<i>ileS</i>	Isoleucyl tRNA synthetase	Mupirocin (bactroban)*	GCTGGATCCTAAGGAGTGAAAAAATGGATTACAAAGAAACG (SEQ ID NO.: 78605); CCGGTCGACCAATTATACAAGTGATTTTACAACTTGTGGCATC (SEQ ID NO.: 78606)
<i>trpS</i>	Tryptophanyl tRNA synthetase	Indolmycin*	GCCGGATCCCTAAGAAAGTAGGCATTTAAATGGAGAC (SEQ ID NO.: 78607); CCGGTCGACGTTTATTTATCTCTTACGTCCTCAAACC (SEQ ID NO.: 78608)
<i>fabF</i>	$\beta$ keto-acyl carrier protein synthase	Cerulenin	GCTGGATCCCAATAGGAGGATAACGAATGAG (SEQ ID NO.: 78609); CAGGTCGACAATTATGCTTCAAATTTCTT (SEQ ID NO.: 78610)

<i>defB</i>	Peptide deformylase	Actinonin	GCTGGATCCATAAGGAAGGTGCAATATATG (SEQ ID NO.: 78611); CAGGTCGACGTTTTAAACTTCTACTGCAT (SEQ ID NO.: 78612)
<i>PBP-2a</i>	Penicillin binding protein 2	Cloxacillin	GCCGGATCCCCAAATGTAGTCTTATATAAGGAGGATATTGATG (SEQ ID NO.: 78613); CAGGTCGACGCGCTTCACCTGTTTGTTCATCTATATC (SEQ ID NO.: 78614)

\* antibiotics unavailable commercially

**EXAMPLE 22****Overexpression of Genes Encoding Gene Products Required for Proliferation Confers Specific Resistance to Antibiotics which Target the Overexpressed Gene Product**

To demonstrate that cells which overexpress a gene encoding a gene product required for proliferation are specifically resistant to antibiotics which target that gene product, the following experiments were performed. Several identical compound plates were prepared as described above in which different antibiotics were present in different wells. Media containing cells overexpressing different genes were separately dispensed into each one of these plates. Plate incubation and growth measurement were the same as described in Example 21 above. Growth was deemed specific if cells overexpressing one particular gene only gained resistance to antibiotics which target the product of the overexpressed gene but not to other antibiotics which target the products of genes which were not overexpressed.

As indicated in Figure 14 overexpression of the *fabI* gene conferred resistance to triclosan, which acts on the gene product of the *fabI* gene, enoyl-acyl carrier protein reductase. However, overexpression of the *fabI* gene did not confer resistance to cerulenin, trimethoprim, or actinonin, each of which act on other gene products.

Similarly, as indicated in Figure 15 overexpression of the *folA* gene conferred resistance to trimethoprim, which acts on the gene product of the *folA* gene, dihydrofolate reductase. However, overexpression of the *folA* gene did not confer resistance to triclosan, cerulenin, or actinonin, each of which act on other gene products.

As indicated in Figure 16 overexpression of the *defB* gene conferred resistance to actinonin, which acts on the gene product of the *defB* gene, peptide deformylase. However, overexpression of the *defB* gene did not confer resistance to cerulenin, trimethoprim, or triclosan, each of which act on other gene products.

As indicated in Figure 17 overexpression of the *fabF* gene conferred resistance to cerulenin, which acts on the gene product of the *fabF* gene,  $\beta$  keto-acyl carrier protein synthase II. However, overexpression of the *fabF* gene did not confer resistance to triclosan, trimethoprim, or actinonin, each of which act on other gene products.

Thus, overexpression of a gene encoding a gene product required for proliferation confers specific resistance to antibiotics which target the overexpressed gene product.

**EXAMPLE 23****Selection of a Strain Overexpressing a Gene Encoding a Target Gene Product from a Mixture of Strains Overexpressing Genes Required for Proliferation**

To confirm that a strain expressing the gene product targeted by an antibiotic can be selected from a mixture of strains which each overexpress a different gene required for



proliferation, the following experiment was performed. *S. aureus* strains overexpressing one of nine genes encoding a gene product required for proliferation were constructed as described above. The nine overexpressed genes were *fabF*, *defB*, *folA*, *fabI*, *ileS*, *fusA*, *gyrB*, *murA*, *rpoB*. A mixture of the nine strains was grown wells in a 96 well plate in medium containing various concentrations of inducer and a sufficient concentration of actinonin, cerulenin, triclosan or trimethoprim to inhibit the growth of strains which do not overexpress the targets of these antibiotics.

Growth was observed in wells containing appropriate inducer concentrations and each one of the four antibiotics (See Figure 18). The cultures which grew in the presence of one of the antibiotics were analyzed as follows. The cultures were removed from the wells of the plate and single colonies were obtained by plating serial dilutions LB agar plates containing an appropriate antibiotic. Plasmids were isolated from at least 60 individual colonies for each culture and the genes which conferred antibiotic resistance were amplified by performing PCR reactions using vector-specific primers. The PCR products were then sequenced.

All of the plasmids obtained from the culture which grew in the presence of cerulenin contained the *fabF* sequence. Similarly, all of the plasmids obtained from clones which grew in the presence of triclosan contained the *fabI* gene. All of the plasmid obtained from colonies which grew in the presence of actinonin contained the *defB* gene. In addition, 81% of the plasmids obtained from colonies which grew in the presence of trimethoprim contained the *folA* gene. Growth conditions could be further optimized to provide 100% recovery of plasmids containing the *folA* gene.

These results demonstrate that a strain expressing the gene product targeted by an antibiotic can be selected from a mixture of strains which each overexpress a different gene required for proliferation.

#### EXAMPLE 24

##### Identification of Amplification Products Having Distinguishable Lengths

The following genes were identified as being required for proliferation as previously described in U.S. Patent Application Serial Number 09/815,242, filed march 21, 2001. Plasmids in which antisense nucleic acids complementary to nucleotide sequences the essential *pbpC*, *secA*, *ylaO(Bs)*, *yphC(Bs)*, *trpS*, *polC*, *fabI*, *rpsR (Bs)*, *fabF(yjaY)*, *ileS*, *murC*, *fnhB*, *murA (Bs)*, *murF(Bs)*, *fisZ*, *tufA*, *gyrA*, *rpoB*, *grlA* or *folA(dfrA)* genes were transcribed from the XylT5 promoter in *Staphylococcus aureus*.

Amplification primers were designed which would yield amplification products of the following lengths if the plasmid encoding the corresponding antisense nucleic acid is present in a mixture of nucleic acids:

	<i>yphC</i>	260bp	<i>secA</i>	267bp
	<i>folA</i>	230 bp	<i>tufA</i>	243bp
	<i>fabI</i>	220bp	<i>gyrA</i>	225bp
	<i>trpS</i>	208bp	<i>ileS</i>	215bp
5	<i>fabF</i>	189bp	<i>murF</i>	203bp
	<i>murA</i>	176bp	<i>fmhB</i>	181bp
	<i>rpoB</i>	159bp	<i>ylaO</i>	169bp
	<i>griA</i>	151bp	<i>pbpC</i>	156bp
	<i>murC</i>	129bp	<i>polC</i>	145bp
10	<i>rpsR</i>	109bp	<i>ftsZ</i>	117bp

The 5' primer of each pair was complementary to a nucleotide sequence within the *xylT5* promoter while 3' primer was complementary to a nucleotide sequence within the antisense clone. The 5' primer of each pair was identical for each amplification reaction. The nucleotide sequence GTTCTT was appended on the 5' end of the 3' primers. One primer in each pair was labeled with either VIC or 6FAM.

Two sets of ten plasmids containing the antisense nucleic acids complementary to the genes listed in each of the columns above were mixed in equal amounts in 11 tubes except that either the plasmid encoding antisense nucleic acids complementary to a nucleotide sequence in the *griA* gene or the plasmid encoding antisense nucleic acids complementary to nucleotide sequences in the *fmhB* gene were serially diluted two fold in each of the 11 tubes (i.e. the first tube had 100pg of the *griA* plasmid or the *fmhB* plasmid while the last tube had 0.10pg of the *griA* plasmid or the *fmhB* plasmid). Amplification reactions were conducted on the mixtures and the amplification products were separated on a 5% NuSieve 3:1 agarose gel (BioWhittaker Molecular Applications Rockland, ME). The levels of the 151bp or 181 amplification products for the *griA* or *fmhB* primer respectively were specifically reduced in a stepwise fashion with increasing dilutions while the levels of the undiluted products remained constant. The assay readily detected a 10-fold decrease in template concentration reflected in the amplification products corresponding to the *griA* or *fmhB* plasmids.

Although this method has been described using examples of antisense nucleic acids to specific essential genes, it will be appreciated that this method can be used with any of the antisense nucleic acids described herein, such as an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, an antisense nucleic acid comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a nucleic acid complementary to a nucleic acid comprising a nucleotide sequence

selected from the group consisting of SEQ ID NOs.: 6214-42397, a nucleic acid complementary to a nucleic acid comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a nucleic acid complementary to a nucleic acid which encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a nucleic acid complementary to a nucleic acid which encodes at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids of a polypeptide sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a homologous antisense nucleic acid, an antisense nucleic acid comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of a homologous nucleic acid, a nucleic acid complementary to a homologous coding nucleic acid, a nucleic acid complementary to at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of a homologous coding nucleic acid, a nucleic acid complementary to a nucleic acid which encodes a homologous polypeptide, or a nucleic acid complementary to a nucleic acid which encodes at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids of a homologous polypeptide. It will also be appreciated that promoters other than XlyT5 can be used to express the gene products described herein. For example, a number of promoters useful for nucleic acid expression (including antisense nucleic acid expression) in *Enterococcus faecalis*, *Staphylococcus aureus* as well as other Gram positive organisms are described in U.S. Patent Application Serial Number 10/032,393, filed December 21, 2001.

Additionally, the above methods can be used with any organism including *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus*

*mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*,  
 5 *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* or any species falling within the genera of any of the above species.

#### EXAMPLE 25

##### 10 Selective Disappearance of Amplification Products Corresponding to Strains Underexpressing a Gene Product on which a Compound which Inhibits Proliferation Acts

Strains of *Staphylococcus aureus* containing plasmids encoding antisense nucleic acids complementary to nucleotide sequences within the *yphC*, *folA*, *fabI*, *trpS*, *fabF*, *murA*, *rpoB*, *griA*, *murC* or *rpsR* genes (described in Example 24 above) were mixed together in identical  
 15 cultures such that the number of cells of each strain in the culture was identical. Each of the cultures containing the ten strains was contacted with one of the following antibiotics at one of the following concentrations:

spectinomycin- 2.5, 5.0ug/ml

mupriocin- 4.3, 8.6, 17.2ug/ml.

20 cerulenin- 4.5, 9.0, 18.0ug/ml

Spectinomycin acts on the product of the *rpsR* gene, mupriocin acts on the product of the *ileS* gene and cerulenin acts on the product of the *FabF* gene. The middle concentration for each antibiotic is its IC50.

The culture containing the ten strains were grown in rich medium (L-Broth; for  
 25 antisense LB + chloroamphenicol to maintain antisense plasmid) until the cells reached early log phase then contacted with of one of the above-stated compounds at one of the concentrations listed above (preferably near IC50). The cultures were grown for a sufficient length of time to permit the compounds to specifically inhibit the growth of strains underexpressing their targets. Preferably the cultures were grown at least 16 hr, more  
 30 preferably between 24 and 48 hrs. It is desirable to avoid allowing the culture to grow for time periods which might place selective pressure on the strains which could lead to false positives.

The cells were harvested by centrifugation and plasmid DNA was isolated from the cultures. PCR amplifications were performed as described in Example 24. Amplification products were run on NuSieve agarose gels as described above. The amounts of the  
 35 amplification products corresponding to each antisense nucleic acid were determined and

5 compared to those in a control culture which was not contacted with the drug or to the amounts of the amplification products corresponding to the other antisense nucleic acids which were not complementary to nucleotide sequences in the genes encoding the gene products on which the compounds act. In each case, only the amplification product corresponding to the target on which the antibiotic acts was not detectable on the gel.

10 It is desirable, in embodiments in which the level or activity of gene products is regulated by transcribing antisense nucleic acids complementary to gene products required for proliferation or by replacing the native promoters of such genes with regulatable promoters, to perform dose-response curve for the inducer used to induce transcription of the antisense nucleic acids or induce transcription from the regulatable promoter. In such embodiments, it is desirable to use the lowest concentration of inducer which provides optimal transcription levels for detecting the effects of a particular test compound while interfering as little as possible with the growth of strains which do not overexpress or underexpress the target on which the compound acts. It also desirable contact the cultures with varying amounts of test compounds  
15 to determine the optimal amounts for obtaining differential growth of strains which overexpress or underexpress the targets on which the compounds act. Preferably, if the strains overexpress gene products required for proliferation, the level of the compound is preferably about  $IC_{90}$  or above. Preferably, if the strains underexpress gene products required for proliferation, the level of the compound is preferably about  $IC_{50}$  or below.

20 It will be appreciated that, if desired, the amplification products may be detected using the dyes described above. It will also be appreciated that amplification products may be detected using any desired amplification method including RT-PCR and PCR. Although this method has been described using examples of antisense nucleic acids to specific essential genes, it will be appreciated that this method can be used with any of the antisense  
25 nucleic acids described herein, such as an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a nucleic acid sequence complementary to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a nucleic acid sequence complementary to a nucleic acid sequence which encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a homologous antisense nucleic acid, a  
30 nucleic acid sequence complementary to a homologous coding nucleic acid, or a nucleic acid complementary to a nucleic acid which encodes a homologous polypeptide. It will also be appreciated that promoters other than *XlyT5* can be used to express the gene products described herein. For example, a number of promoters useful for nucleic acid expression (including  
35 antisense nucleic acid expression) in *Enterococcus faecalis*, *Staphylococcus aureus* as well as

other Gram positive organisms are described in U.S. Patent Application Serial Number 10/032,393, filed December 21, 2001.

Additionally, the above methods can be used with any organism including *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*,  
 5 *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*,  
*Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*,  
*Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*,  
*Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*),  
*Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium*  
 10 *acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*,  
*Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter*  
*cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus*  
*influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella*  
*pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*,  
 15 *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma*  
*genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia*  
*asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus*  
*mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas*  
*syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella*  
 20 *paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*,  
*Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*,  
*Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus*  
*pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio*  
*parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* or any species  
 25 falling within the genera of any of the above species.

#### EXAMPLE 26

##### Use of Identified Nucleic Acid Sequences as Probes

The sequences from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*,  
*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter*  
 30 *baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*,  
*Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*,  
*Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium*  
*botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*,  
*Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*,  
 35 *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*,

*Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*,  
5 *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* described herein, homologous coding nucleic acids, or homologous antisense nucleic acids can be used as probes to obtain the sequence of additional genes of interest from a second cell or microorganism. For example, probes to genes encoding potential bacterial target proteins may be hybridized to nucleic acids from other  
10 organisms including other bacteria and higher organisms, to identify homologous sequences in these other organisms. For example, the identified sequences from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*,  
15 *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*,  
20 *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or  
25 *Yersinia pestis*, homologous coding nucleic acids, or homologous antisense nucleic acids may be used to identify homologous sequences in *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*),  
30 *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*,  
35 *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*,

*Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*,  
 5 *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*,  
 10 *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* or any species falling within the genera of any of the above species. In some embodiments of the present invention, the nucleic acids from  
 15 *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*,  
 20 *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*,  
 25 *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* described herein, homologous coding nucleic acids, or homologous antisense nucleic acids may be used to identify homologous nucleic acids from a heterologous organism other than *E. coli*.

30 Hybridization between the nucleic acids from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*,  
 35



*Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*,  
*Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium*  
*avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*,  
*Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria*  
5 *meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas*  
*syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus*  
*haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*,  
*Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* described  
herein, homologous coding nucleic acids, or homologous antisense nucleic acids and nucleic acids  
10 from humans might indicate that the protein encoded by the gene to which the probe corresponds is  
found in humans and therefore not necessarily an optimal drug target. Alternatively, the gene can  
be conserved only in bacteria and therefore would be a good drug target for a broad spectrum  
antibiotic or antimicrobial. These probes can also be used in a known manner to isolate  
homologous nucleic acids from *Staphylococcus*, *Salmonella*, *Klebsiella*, *Pseudomonas*,  
15 *Enterococcus* or other cells or microorganisms, e.g. by screening a genomic or cDNA library.

Probes derived from the nucleic acid sequences from *Escherichia coli*, *Staphylococcus*  
*aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella*  
*typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella*  
*pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia*  
20 *mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium*  
*acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*,  
*Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*,  
*Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium*  
*avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*,  
25 *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria*  
*meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas*  
*syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus*  
*haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*,  
*Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* described  
30 herein, homologous coding nucleic acids, or homologous antisense nucleic acids, or portions  
thereof, can be labeled with detectable labels familiar to those skilled in the art, including  
radioisotopes and non-radioactive labels, to provide a detectable probe. The detectable probe can  
be single stranded or double stranded and can be made using techniques known in the art, including  
*in vitro* transcription, nick translation, or kinase reactions. A nucleic acid sample containing a  
35 sequence capable of hybridizing to the labeled probe is contacted with the labeled probe. If the

nucleic acid in the sample is double stranded, it can be denatured prior to contacting the probe. In some applications, the nucleic acid sample can be immobilized on a surface such as a nitrocellulose or nylon membrane. The nucleic acid sample can comprise nucleic acids obtained from a variety of sources, including genomic DNA, cDNA libraries, RNA, or tissue samples.

5           Procedures used to detect the presence of nucleic acids capable of hybridizing to the detectable probe include well known techniques such as Southern blotting, Northern blotting, dot blotting, colony hybridization, and plaque hybridization. In some applications, the nucleic acid capable of hybridizing to the labeled probe can be cloned into vectors such as expression vectors, sequencing vectors, or in vitro transcription vectors to facilitate the characterization and expression  
10           of the hybridizing nucleic acids in the sample. For example, such techniques can be used to isolate, purify and clone sequences from a genomic library, made from a variety of bacterial species, which are capable of hybridizing to probes made from the sequences identified as described herein.

#### EXAMPLE 27

##### Preparation of PCR Primers and Amplification of DNA

15           The identified *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium*  
20           *botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus*  
25           *mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* genes corresponding directly to or located within the operon of nucleic acid sequences required for proliferation, homologous coding nucleic acids,  
30           or homologous antisense nucleic acids or portions thereof can be used to prepare PCR primers for a variety of applications, including the identification or isolation of homologous sequences from other species. For example, the *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*,  
35           *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*,

*Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*,  
 5 *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* genes may be used to prepare PCR primers to  
 10 identify or isolate homologous sequences from *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*),  
 15 *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*,  
 20 *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*,  
 25 *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*,  
 30 *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* or any species falling within the genera of any of the above species. In some embodiments of the present invention, the PCR primers may be used to identify or isolate homologous nucleic acids from an organism other than *E. coli*.

The identified or isolated nucleic acids obtained using the PCR primers may contain part or all of the homologous nucleic acids. Because homologous nucleic acids are related but not identical in sequence, those skilled in the art will often employ degenerate sequence PCR primers. Such degenerate sequence primers are designed based on sequence regions that are either known to be conserved or suspected to be conserved such as conserved coding regions. The successful production of a PCR product using degenerate probes generated from the sequences identified herein would indicate the presence of a homologous gene sequence in the species being screened. The PCR primers are at least 10 nucleotides, and preferably at least 20 nucleotides in length. More preferably, the PCR primers are at least 20-30 nucleotides in length. In some embodiments, the PCR primers can be more than 30 nucleotides in length. It is preferred that the primer pairs have approximately the same G/C ratio, so that melting temperatures are approximately the same. A variety of PCR techniques are familiar to those skilled in the art. For a review of PCR technology, see Molecular Cloning to Genetic Engineering White, B.A. Ed. in **Methods in Molecular Biology** 67: Humana Press, Totowa 1997. When the entire coding sequence of the target gene is known, the 5' and 3' regions of the target gene can be used as the sequence source for PCR probe generation. In each of these PCR procedures, PCR primers on either side of the nucleic acid sequences to be amplified are added to a suitably prepared nucleic acid sample along with dNTPs and a thermostable polymerase such as Taq polymerase, Pfu polymerase, or Vent polymerase. The nucleic acid in the sample is denatured and the PCR primers are specifically hybridized to complementary nucleic acid sequences in the sample. The hybridized primers are extended. Thereafter, another cycle of denaturation, hybridization, and extension is initiated. The cycles are repeated multiple times to produce an amplified fragment containing the nucleic acid sequence between the primer sites.

#### EXAMPLE 28

##### Inverse PCR

The technique of inverse polymerase chain reaction can be used to extend the known nucleic acid sequence identified as described herein. The inverse PCR reaction is described generally by Ochman et al., in Ch. 10 of **PCR Technology: Principles and Applications for DNA Amplification**, (Henry A. Erlich, Ed.) W.H. Freeman and Co. (1992). Traditional PCR requires two primers that are used to prime the synthesis of complementary strands of DNA. In inverse PCR, only a core sequence need be known.

Using the sequences identified as relevant from the techniques taught in Examples 10 and 11 and applied to other species of bacteria, a subset of nucleic sequences are identified that correspond to genes or operons that are required for bacterial proliferation. In species for which a genome sequence is not known, the technique of inverse PCR provides a method for obtaining the

gene in order to determine the sequence or to place the probe sequences in full context to the target sequence to which the identified nucleic acid sequence binds.

To practice this technique, the genome of the target organism is digested with an appropriate restriction enzyme so as to create fragments of nucleic acid that contain the identified sequence as well as unknown sequences that flank the identified sequence. These fragments are then circularized and become the template for the PCR reaction. PCR primers are designed in accordance with the teachings of Example 27 and directed to the ends of the identified sequence.. The primers direct nucleic acid synthesis away from the known sequence and toward the unknown sequence contained within the circularized template. After the PCR reaction is complete, the resulting PCR products can be sequenced so as to extend the sequence of the identified gene past the core sequence of the identified exogenous nucleic acid sequence identified. In this manner, the full sequence of each novel gene can be identified. Additionally the sequences of adjacent coding and noncoding regions can be identified.

#### EXAMPLE 29

##### Identification of Genes Required for *Escherichia coli* Proliferation

Genes required for proliferation in *Escherichia coli* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

#### EXAMPLE 30

##### Identification of Genes Required for *Staphylococcus aureus* Proliferation

Genes required for proliferation in *Staphylococcus aureus* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

#### EXAMPLE 31

##### Identification of Genes Required for *Enterococcus faecalis* Proliferation

Genes required for proliferation in *Enterococcus faecalis* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

#### EXAMPLE 32

##### Identification of Genes Required for *Klebsiella pneumoniae* Proliferation

Genes required for proliferation in *Klebsiella pneumoniae* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 33****Identification of Genes Required for *Pseudomonas aeruginosa* Proliferation**

Genes required for proliferation in *Pseudomonas aeruginosa* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 34****Identification of Genes Required for *Salmonella typhimurium* Proliferation**

Genes required for proliferation in *Salmonella typhimurium* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 35****Identification of Genes Required for *Acinetobacter baumannii* Proliferation**

Genes required for proliferation in *Acinetobacter baumannii* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 36****Identification of Genes Required for *Bacillus anthracis* Proliferation**

Genes required for proliferation in *Bacillus anthracis* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 37****Identification of Genes Required for *Bordetella pertussis* Proliferation**

Genes required for proliferation in *Bordetella pertussis* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 38****Identification of Genes Required for *Borrelia burgdorferi* Proliferation**

Genes required for proliferation in *Borrelia burgdorferi* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 39****Identification of Genes Required for *Burkholderia cepacia* Proliferation**

Genes required for proliferation in *Burkholderia cepacia* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 40****Identification of Genes Required for *Burkholderia fungorum* Proliferation**

Genes required for proliferation in *Burkholderia fungorum* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
5 identify essential genes described herein.

**EXAMPLE 41****Identification of Genes Required for *Burkholderia mallei* Proliferation**

Genes required for proliferation in *Burkholderia mallei* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
10 identify essential genes described herein.

**EXAMPLE 42****Identification of Genes Required for *Campylobacter jejuni* Proliferation**

Genes required for proliferation in *Campylobacter jejuni* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
15 identify essential genes described herein.

**EXAMPLE 43****Identification of Genes Required for *Chlamydia pneumoniae* Proliferation**

Genes required for proliferation in *Chlamydia pneumoniae* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
20 identify essential genes described herein.

**EXAMPLE 44****Identification of Genes Required for *Chlamydia trachomatis* Proliferation**

Genes required for proliferation in *Chlamydia trachomatis* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
25 identify essential genes described herein.

**EXAMPLE 45****Identification of Genes Required for *Clostridium acetobutylicum* Proliferation**

Genes required for proliferation in *Clostridium acetobutylicum* are identified according to the methods described above. For example, promoters and vectors described herein can be used  
30 to identify essential genes described herein.

**EXAMPLE 46****Identification of Genes Required for *Clostridium botulinum* Proliferation**

Genes required for proliferation in *Clostridium botulinum* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
35 identify essential genes described herein.

**EXAMPLE 47****Identification of Genes Required for *Clostridium difficile* Proliferation**

Genes required for proliferation in *Clostridium difficile* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
5 identify essential genes described herein.

**EXAMPLE 48****Identification of Genes Required for *Corynebacterium diphtheriae* Proliferation**

Genes required for proliferation in *Corynebacterium diphtheriae* are identified according to the methods described above. For example, promoters and vectors described herein can be  
10 used to identify essential genes described herein.

**EXAMPLE 49****Identification of Genes Required for *Enterobacter cloacae* Proliferation**

Genes required for proliferation in *Enterobacter cloacae* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
15 identify essential genes described herein.

**EXAMPLE 50****Identification of Genes Required for *Enterococcus faecium* Proliferation**

Genes required for proliferation in *Enterococcus faecium* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
20 identify essential genes described herein.

**EXAMPLE 51****Identification of Genes Required for *Haemophilus influenzae* Proliferation**

Genes required for proliferation in *Haemophilus influenzae* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
25 identify essential genes described herein.

**EXAMPLE 52****Identification of Genes Required for *Helicobacter pylori* Proliferation**

Genes required for proliferation in *Helicobacter pylori* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
30 identify essential genes described herein.

**EXAMPLE 53****Identification of Genes Required for *Legionella pneumophila* Proliferation**

Genes required for proliferation in *Legionella pneumophila* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
35 identify essential genes described herein.



**EXAMPLE 54****Identification of Genes Required for *Listeria monocytogenes* Proliferation**

Genes required for proliferation in *Listeria monocytogenes* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
5 identify essential genes described herein.

**EXAMPLE 55****Identification of Genes Required for *Moraxella catarrhalis* Proliferation**

Genes required for proliferation in *Moraxella catarrhalis* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
10 identify essential genes described herein.

**EXAMPLE 56****Identification of Genes Required for *Mycobacterium avium* Proliferation**

Genes required for proliferation in *Mycobacterium avium* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
15 identify essential genes described herein.

**EXAMPLE 57****Identification of Genes Required for *Mycobacterium bovis* Proliferation**

Genes required for proliferation in *Mycobacterium bovis* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
20 identify essential genes described herein.

**EXAMPLE 58****Identification of Genes Required for *Mycobacterium leprae* Proliferation**

Genes required for proliferation in *Mycobacterium leprae* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
25 identify essential genes described herein.

**EXAMPLE 59****Identification of Genes Required for *Mycobacterium tuberculosis* Proliferation**

Genes required for proliferation in *Mycobacterium tuberculosis* are identified according to the methods described above. For example, promoters and vectors described herein can be  
30 used to identify essential genes described herein.

**EXAMPLE 60****Identification of Genes Required for *Mycoplasma genitalium* Proliferation**

Genes required for proliferation in *Mycoplasma genitalium* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
35 identify essential genes described herein.

**EXAMPLE 61****Identification of Genes Required for *Mycoplasma pneumoniae* Proliferation**

Genes required for proliferation in *Mycoplasma pneumoniae* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 62****Identification of Genes Required for *Neisseria gonorrhoeae* Proliferation**

Genes required for proliferation in *Neisseria gonorrhoeae* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 63****Identification of Genes Required for *Neisseria meningitidis* Proliferation**

Genes required for proliferation in *Neisseria meningitidis* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 64****Identification of Genes Required for *Pasteurella multocida* Proliferation**

Genes required for proliferation in *Pasteurella multocida* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 65****Identification of Genes Required for *Proteus mirabilis* Proliferation**

Genes required for proliferation in *Proteus mirabilis* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 66****Identification of Genes Required for *Pseudomonas putida* Proliferation**

Genes required for proliferation in *Pseudomonas putida* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 67****Identification of Genes Required for *Pseudomonas syringae* Proliferation**

Genes required for proliferation in *Pseudomonas syringae* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 68****Identification of Genes Required for *Salmonella paratyphi* Proliferation**

Genes required for proliferation in *Salmonella paratyphi* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
5 identify essential genes described herein.

**EXAMPLE 69****Identification of Genes Required for *Salmonella typhi* Proliferation**

Genes required for proliferation in *Salmonella typhi* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
10 identify essential genes described herein.

**EXAMPLE 70****Identification of Genes Required for *Staphylococcus epidermidis* Proliferation**

Genes required for proliferation in *Staphylococcus epidermidis* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
15 identify essential genes described herein.

**EXAMPLE 71****Identification of Genes Required for *Staphylococcus haemolyticus* Proliferation**

Genes required for proliferation in *Staphylococcus haemolyticus* are identified according to the methods described above. For example, promoters and vectors described herein can be  
20 used to identify essential genes described herein.

**EXAMPLE 72****Identification of Genes Required for *Streptococcus mutans* Proliferation**

Genes required for proliferation in *Streptococcus mutans* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
25 identify essential genes described herein.

**EXAMPLE 73****Identification of Genes Required for *Streptococcus pneumoniae* Proliferation**

Genes required for proliferation in *Streptococcus pneumoniae* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
30 identify essential genes described herein.

**EXAMPLE 74****Identification of Genes Required for *Streptococcus pyogenes* Proliferation**

Genes required for proliferation in *Streptococcus pyogenes* are identified according to the methods described above. For example, promoters and vectors described herein can be used to  
35 identify essential genes described herein.

**EXAMPLE 75****Identification of Genes Required for *Treponema pallidum* Proliferation**

Genes required for proliferation in *Treponema pallidum* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 76****Identification of Genes Required for *Ureaplasma urealyticum* Proliferation**

Genes required for proliferation in *Ureaplasma urealyticum* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 77****Identification of Genes Required for *Vibrio cholerae* Proliferation**

Genes required for proliferation in *Vibrio cholerae* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 78****Identification of Genes Required for *Yersinia pestis* Proliferation**

Genes required for proliferation in *Yersinia pestis* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 79****Identification of Genes Required for *Salmonella enterica* Proliferation**

Genes required for proliferation in *Salmonella enterica* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 80****Identification of Genes Required for *Aspergillus fumigatus* Proliferation**

Genes required for proliferation in *Aspergillus fumigatus* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 81****Identification of Genes Required for *Plasmodium ovale* Proliferation**

Genes required for proliferation in *Plasmodium ovale* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 82****Identification of Genes Required for *Entamoeba histolytica* Proliferation**

Genes required for proliferation in *Entamoeba histolytica* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 83****Identification of Genes Required for *Candida albicans* Proliferation**

Genes required for proliferation in *Candida albicans* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 84****Identification of Genes Required for *Histoplasma capsulatum* Proliferation**

Genes required for proliferation in *Histoplasma capsulatum* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**EXAMPLE 85****Identification of Genes Required for *Salmonella choleraesuis* Proliferation**

Genes required for proliferation in *Salmonella choleraesuis* are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

**Use of Isolated Exogenous Nucleic Acid Fragments as Antisense Antibiotics**

In addition to using the identified sequences to enable screening of molecule libraries to identify compounds useful to identify antibiotics, antisense nucleic acids complementary to the proliferation-required sequences or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids, or homologous antisense nucleic acids can be used as therapeutic agents. Specifically, the proliferation-required sequences or homologous coding nucleic acids, or portions thereof, in an antisense orientation or homologous antisense nucleic acids can be provided to an individual to inhibit the translation of a bacterial target gene or the processing, folding, or assembly into a protein/RNA complex of a nontranslated RNA.

**EXAMPLE 86****Generation of Antisense Therapeutics from Identified Exogenous Sequences**

Antisense nucleic acids complementary to the proliferation-required sequences described herein, or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids, or portions thereof, or homologous antisense nucleic acids or portions thereof can be used as antisense therapeutics for the treatment of bacterial infections or simply

for inhibition of bacterial growth *in vitro* or *in vivo*. For example, the antisense therapeutics may be used to treat bacterial infections caused by *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or to inhibit the growth of these organisms. The antisense therapeutics may also be used to treat infections caused by or to inhibit the growth of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*,

*Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* or any species falling within the genera of any of the above species. In some embodiments of the present invention, the antisense therapeutics may be used to treat infection by or inhibit the growth of an organism other than *E. coli*.

The therapy exploits the biological process in cells where genes are transcribed into messenger RNA (mRNA) that is then translated into proteins. Antisense RNA technology contemplates the use of antisense nucleic acids, including antisense oligonucleotides, complementary to a target gene that will bind to its target nucleic acid and decrease or inhibit the expression of the target gene. For example, the antisense nucleic acid may inhibit the translation or transcription of the target nucleic acid. In one embodiment, antisense oligonucleotides can be used to treat and control a bacterial infection of a cell culture containing a population of desired cells contaminated with bacteria. In another embodiment, the antisense oligonucleotides can be used to treat an organism with a bacterial infection.

Antisense oligonucleotides can be synthesized from any of the sequences of the present invention using methods well known in the art. In a preferred embodiment, antisense oligonucleotides are synthesized using artificial means. Uhlmann & Peymann, Chemical Rev. 90:543-584 (1990) review antisense oligonucleotide technology in detail. Modified or unmodified antisense oligonucleotides can be used as therapeutic agents. Modified antisense oligonucleotides are preferred. Modification of the phosphate backbones of the antisense oligonucleotides can be achieved by substituting the internucleotide phosphate residues with methylphosphonates, phosphorothioates, phosphoramidates, and phosphate esters. Nonphosphate internucleotide analogs such as siloxane bridges, carbonate bridges, thioester bridges, as well as many others known in the art may also be used. The preparation of certain antisense oligonucleotides with modified internucleotide linkages is described in U.S. Patent No. 5,142,047.

Modifications to the nucleoside units of the antisense oligonucleotides are also contemplated. These modifications can increase the half-life and increase cellular rates of uptake for the oligonucleotides *in vivo*. For example,  $\alpha$ -anomeric nucleotide units and modified nucleotides such as 1,2-dideoxy-d-ribofuranose, 1,2-dideoxy-1-phenylribofuranose, and  $N^4$ ,  $N^4$ -ethano-5-methyl-cytosine are contemplated for use in the present invention.

An additional form of modified antisense molecules is found in peptide nucleic acids. Peptide nucleic acids (PNA) have been developed to hybridize to single and double stranded nucleic acids. PNA are nucleic acid analogs in which the entire deoxyribose-phosphate backbone

has been exchanged with a chemically different, but structurally homologous, polyamide (peptide) backbone containing 2-aminoethyl glycine units. Unlike DNA, which is highly negatively charged, the PNA backbone is neutral. Therefore, there is much less repulsive energy between complementary strands in a PNA-DNA hybrid than in the comparable DNA-DNA hybrid, and consequently they are much more stable. PNA can hybridize to DNA in either a Watson/Crick or Hoogsteen fashion (Demidov et al., *Proc. Natl. Acad. Sci. U.S.A.* 92:2637-2641, 1995; Egholm, *Nature* 365:566-568, 1993; Nielsen et al., *Science* 254:1497-1500, 1991; Dueholm et al., *New J. Chem.* 21:19-31, 1997).

Molecules called PNA "clamps" have been synthesized which have two identical PNA sequences joined by a flexible hairpin linker containing three 8-amino-3,6-dioxaoctanoic acid units. When a PNA clamp is mixed with a complementary homopurine or homopyrimidine DNA target sequence, a PNA-DNA-PNA triplex hybrid can form which has been shown to be extremely stable (Bentin et al., *Biochemistry* 35:8863-8869, 1996; Egholm et al., *Nucleic Acids Res.* 23:217-222, 1995; Griffith et al., *J. Am. Chem. Soc.* 117:831-832, 1995).

The sequence-specific and high affinity duplex and triplex binding of PNA have been extensively described (Nielsen et al., *Science* 254:1497-1500, 1991; Egholm et al., *J. Am. Chem. Soc.* 114:9677-9678, 1992; Egholm et al., *Nature* 365:566-568, 1993; Almarsson et al., *Proc. Natl. Acad. Sci. U.S.A.* 90:9542-9546, 1993; Demidov et al., *Proc. Natl. Acad. Sci. U.S.A.* 92:2637-2641, 1995). They have also been shown to be resistant to nuclease and protease digestion (Demidov et al., *Biochem. Pharm.* 48:1010-1313, 1994). PNA has been used to inhibit gene expression (Hanvey et al., *Science* 258:1481-1485, 1992; Nielsen et al., *Nucl. Acids. Res.*, 21:197-200, 1993; Nielsen et al., *Gene* 149:139-145, 1994; Good & Nielsen, *Science*, 95: 2073-2076, 1998; to block restriction enzyme activity (Nielsen et al., *supra.*, 1993), to act as an artificial transcription promoter (Mollegaard, *Proc. Natl. Acad. Sci. U.S.A.* 91:3892-3895, 1994) and as a pseudo restriction endonuclease (Demidov et al., *Nucl. Acids. Res.* 21:2103-2107, 1993). Recently, PNA has also been shown to have antiviral and antitumoral activity mediated through an antisense mechanism (Norton, *Nature Biotechnol.*, 14:615-619, 1996; Hirschman et al., *J. Investig. Med.* 44:347-351, 1996). PNAs have been linked to various peptides in order to promote PNA entry into cells (Basu et al., *Bioconj. Chem.* 8:481-488, 1997; Pardridge et al., *Proc. Natl. Acad. Sci. U.S.A.* 92:5592-5596, 1995).

The antisense oligonucleotides contemplated by the present invention can be administered by direct application of oligonucleotides to a target using standard techniques well known in the art. The antisense oligonucleotides can be generated within the target using a plasmid, or a phage. Alternatively, the antisense nucleic acid may be expressed from a sequence in the chromosome of the target cell. For example, a promoter may be introduced into



the chromosome of the target cell near the target gene such that the promoter directs the transcription of the antisense nucleic acid. Alternatively, a nucleic acid containing the antisense sequence operably linked to a promoter may be introduced into the chromosome of the target cell. It is further contemplated that the antisense oligonucleotides are incorporated in a ribozyme sequence to enable the antisense to specifically bind and cleave its target mRNA. For technical applications of ribozyme and antisense oligonucleotides see Rossi et al., *Pharmacol. Ther.* 50(2):245-254, (1991). The present invention also contemplates using a retron to introduce an antisense oligonucleotide to a cell. Retron technology is exemplified by U.S. Patent No. 5,405,775. Antisense oligonucleotides can also be delivered using liposomes or by electroporation techniques which are well known in the art.

The antisense nucleic acids described above can also be used to design antibiotic compounds comprising nucleic acids which function by intracellular triple helix formation. Triple helix oligonucleotides are used to inhibit transcription from a genome. The antisense nucleic acids can be used to inhibit cell or microorganism gene expression in individuals infected with such microorganisms or containing such cells. Traditionally, homopurine sequences were considered the most useful for triple helix strategies. However, homopyrimidine sequences can also inhibit gene expression. Such homopyrimidine oligonucleotides bind to the major groove at homopurine:homopyrimidine sequences. Thus, both types of sequences based on the sequences from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or homologous nucleic acids that are required for proliferation are contemplated for use as antibiotic compound templates.

The antisense nucleic acids, such as antisense oligonucleotides, which are complementary to the proliferation-required nucleic acids from *Escherichia coli*,

*Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*,  
 5 *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or to homologous coding nucleic acids, or portions thereof, may be used to  
 10 induce bacterial cell death or at least bacterial stasis by inhibiting target nucleic acid transcription or translation. Antisense oligonucleotides complementary to about 8 to 40 nucleotides of the proliferation-required nucleic acids described herein or homologous coding nucleic acids have sufficient complementarity to form a duplex with the target sequence under physiological conditions.

20 To kill bacterial cells or inhibit their growth, the antisense oligonucleotides are applied to the bacteria or to the target cells under conditions that facilitate their uptake. These conditions include sufficient incubation times of cells and oligonucleotides so that the antisense oligonucleotides are taken up by the cells. In one embodiment, an incubation period of 7-10 days is sufficient to kill bacteria in a sample. An optimum concentration of antisense  
 25 oligonucleotides is selected for use.

The concentration of antisense oligonucleotides to be used can vary depending on the type of bacteria sought to be controlled, the nature of the antisense oligonucleotide to be used, and the relative toxicity of the antisense oligonucleotide to the desired cells in the treated culture. Antisense oligonucleotides can be introduced to cell samples at a number of different  
 30 concentrations preferably between  $1 \times 10^{-10}$  M to  $1 \times 10^{-4}$  M. Once the minimum concentration that can adequately control gene expression is identified, the optimized dose is translated into a dosage suitable for use *in vivo*. For example, an inhibiting concentration in culture of  $1 \times 10^{-7}$  translates into a dose of approximately 0.6 mg/kg body weight. Levels of oligonucleotide approaching 100 mg/kg body weight or higher may be possible after testing the toxicity of the oligonucleotide in laboratory  
 35 animals. It is additionally contemplated that cells from the subject are removed, treated with the

antisense oligonucleotide, and reintroduced into the subject. This range is merely illustrative and one of skill in the art are able to determine the optimal concentration to be used in a given case.

After the bacterial cells have been killed or controlled in a desired culture, the desired cell population may be used for other purposes.

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#### EXAMPLE 87

##### Use of Antisense Oligonucleotides to Treat Contaminated Cell Cultures

The following example demonstrates the ability of an *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* antisense oligonucleotide or an antisense oligonucleotide complementary to a homologous coding nucleic acid, or portions thereof, to act as a bacteriocidal or bacteriostatic agent to treat a contaminated cell culture system. The application of the antisense oligonucleotides of the present invention are thought to inhibit the translation of bacterial gene products required for proliferation. The antisense nucleic acids may also inhibit the transcription, folding or processing of the target RNA.

In one embodiment of the present invention, the antisense oligonucleotide may comprise a phosphorothioate modified nucleic acid comprising at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, or more than 40 consecutive nucleotides of an antisense nucleic acid listed in Table IA (SEQ ID NOs.: 1-6213). A sense oligodeoxynucleotide complementary to the antisense sequence is synthesized and used as a control. The oligonucleotides are synthesized and purified according to the procedures of Matsukura, et al., Gene 72:343 (1988). The test oligonucleotides are dissolved in a small volume of autoclaved water and added to culture medium to make a 100 micromolar stock solution.

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Human bone marrow cells are obtained from the peripheral blood of two patients and cultured according standard procedures well known in the art. The culture is contaminated with *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or an organism containing a homologous nucleic acid and incubated at 37°C overnight to establish bacterial infection.

The control and antisense oligonucleotide containing solutions are added to the contaminated cultures and monitored for bacterial growth. After a 10 hour incubation of culture and oligonucleotides, samples from the control and experimental cultures are drawn and analyzed for the translation of the target bacterial gene using standard microbiological techniques well known in the art. The target *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* gene or an

organism containing the homologous coding nucleic acid is found to be translated in the control culture treated with the control oligonucleotide, however, translation of the target gene in the experimental culture treated with the antisense oligonucleotide of the present invention is not detected or reduced, indicating that the culture is no longer contaminated or is contaminated at a reduced level.

#### EXAMPLE 88

##### Use of Antisense Oligonucleotides to Treat Infections

A subject suffering from a *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Yersinia pestis* infection or an infection with an organism containing a homologous coding nucleic acid is treated with the antisense oligonucleotide preparation above. The antisense oligonucleotide is provided in a pharmaceutically acceptable carrier at a concentration effective to inhibit the transcription or translation of the target nucleic acid. The present subject is treated with a concentration of antisense oligonucleotide sufficient to achieve a blood concentration of about 0.1-100 micromolar. The patient receives daily injections of antisense oligonucleotide to maintain this concentration for a period of 1 week. At the end of the week a blood sample is drawn and analyzed for the presence or absence of the organism using standard techniques well known in the art. There is no detectable evidence of *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*,

*Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Yersinia pestis* or an organism containing a homologous coding nucleic acid and the treatment is terminated.

Antisense nucleic acids complementary to a homologous coding nucleic acid or a portion thereof may be used in the preceding method to treat individuals infected with an organism containing the homologous coding nucleic acid.

#### EXAMPLE 89

##### Preparation and Use of Triple Helix Forming Oligonucleotides

The sequences of proliferation-required nucleic acids, homologous coding nucleic acids, or homologous antisense nucleic acids are scanned to identify 10-mer to 20-mer homopyrimidine or homopurine stretches that could be used in triple-helix based strategies for inhibiting gene expression. Following identification of candidate homopyrimidine or homopurine stretches, their efficiency in inhibiting gene expression is assessed by introducing varying amounts of oligonucleotides containing the candidate sequences into a population of bacterial cells that normally express the target gene. The oligonucleotides may be prepared on an oligonucleotide synthesizer or they may be purchased commercially from a company specializing in custom oligonucleotide synthesis.

The oligonucleotides can be introduced into the cells using a variety of methods known to those skilled in the art, including but not limited to calcium phosphate precipitation, DEAE-Dextran, electroporation, liposome-mediated transfection or native uptake.

Treated cells are monitored for a reduction in proliferation using techniques such as monitoring growth levels as compared to untreated cells using optical density measurements. The oligonucleotides that are effective in inhibiting gene expression in cultured cells can then be introduced *in vivo* using the techniques well known in that art at a dosage level shown to be effective.

In some embodiments, the natural (beta) anomers of the oligonucleotide units can be replaced with alpha anomers to render the oligonucleotide more resistant to nucleases. Further, an intercalating agent such as ethidium bromide, or the like, can be attached to the 3' end of the alpha oligonucleotide to stabilize the triple helix. For information on the generation of oligonucleotides

suitable for triple helix formation see Griffin et al. (Science 245:967-971 (1989), which is hereby incorporated by this reference).

#### EXAMPLE 90

##### Identification of Bacterial Strains from Isolated Specimens by PCR

5            Classical bacteriological methods for the detection of various bacterial species are time consuming and costly. These methods include growing the bacteria isolated from a subject in specialized medium, cultivation on selective agar medium, followed by a set of confirmation assays that can take from 8 to 10 days or longer to complete. Use of the identified sequences of the present invention provides a method to dramatically reduce the time necessary to detect and  
10          identify specific bacterial species present in a sample.

            In one exemplary method, bacteria are grown in enriched medium and DNA samples are isolated from specimens of, for example, blood, urine, stool, saliva or central nervous system fluid by conventional methods. A panel of PCR primers based on identified sequences unique to various species or types of cells or microorganisms are then utilized in accordance with Example 27 to  
15          amplify DNA of approximately 100-200 nucleotides in length from the specimen. A separate PCR reaction is set up for each pair of PCR primers and after the PCR reaction is complete, the reaction mixtures are assayed for the presence of PCR product. The presence or absence of bacteria from the species to which the PCR primer pairs belong is determined by the presence or absence of a PCR product in the various test PCR reaction tubes.

20          Although the PCR reaction is used to assay the isolated sample for the presence of various bacterial species, other assays such as the Southern blot hybridization are also contemplated.

            Compounds which inhibit the activity or reduce the amount of gene products required for proliferation may be identified using rational drug design. These methods may be used with the proliferation-required polypeptides described herein or homologous polypeptides. In such  
25          methods, the structure of the gene product is determined using methods such as x-ray crystallography, NMR, or computer modelling. Compounds are screened to identify those which have a structure which allows them to interact with the gene product. In some embodiments, the compounds are screened to identify those which have structures which allow them to interact with regions of the gene product which are important for its activity. For example, the compounds may  
30          be screened to identify those which have structures which allow them to bind to the active site of the gene product to inhibit its activity. For example, the compound may be a suicide substrate which binds to the active site with high affinity, thereby preventing the gene product from acting on its natural substrate. Alternatively, the compound may bind to a region of the gene product which is involved in complex formation with other biomolecules. In such instances, the activity of the

gene product is inhibited by blocking the interaction between the gene product and other members of the complex.

Thus, one embodiment of the present invention comprises a method of using a crystal of the gene products of the present invention and/or a dataset comprising the three-dimensional coordinates obtained from the crystal in a drug-screening assay. The present invention also includes agents (modulators or drugs) that are identified by the methods of the present invention, along with the method of using agents (modulators or drugs) identified by a method of the present invention, for inhibiting the activity of or modulating the amount of an essential gene product. The present invention also includes crystals comprising the gene products of the present invention or portions thereof.

In some embodiments of the present invention, the three-dimensional structure of the polypeptides required for proliferation is determined using X-ray crystallography or NMR. The coordinates of the determined structure are used in computer-assisted modeling programs to identify compounds that bind to and/or modulate the activity or amount of the encoded polypeptide. The method may include the following steps: 1) the generation of high-purity crystals of the encoded recombinant (or endogenous) polypeptide for analysis; 2) determination of the three-dimensional structure of the polypeptide; and, 3) the use of computer-assisted "docking" programs to analyze the molecular interaction of compound structure and the polypeptide (i.e., drug screening).

General methods for performing each of the above steps are described below and are also well known to those of skill in the art. Any method known to those of skill in the art, including those described herein, may be employed for generating the three-dimensional structure for each identified essential gene product and its use in the drug-screening assays.

Crystals of the gene products required for proliferation may be obtained as follows. Under certain conditions, molecules condense from solution into a highly-ordered crystalline lattice, which is defined by a unit cell, the smallest repeating volume of the crystalline array. The contents of such a cell can interact with and diffract certain electromagnetic and particle waves (e.g., X-rays, neutron beams, electron beams etc.). Due to the symmetry of the lattice, the diffracted waves interact to create a diffraction pattern. By measuring the diffraction pattern, crystallographers are able to reconstruct the three-dimensional structure of the atoms in the crystal.

Any method known to those of skill in the art, including those set forth below, may be employed to prepare high-purity crystals. For example, crystals of the product of the identified essential gene can be grown by a number of techniques including batch crystallization, vapor diffusion (either by sitting drop or hanging drop) and by microdialysis. Seeding of the crystals



in some instances is required to obtain X-ray quality crystals. Standard micro and/or macro seeding of crystals may therefore be used. Exemplified below is the hanging-drop vapor diffusion procedure. Hanging drops of an essential gene product (2.5  $\mu$ l, 10 mg/ml) in 20 mM Tris, pH=8.0, 100 mM NaCl are mixed with an equal amount of reservoir buffer containing 2.7-3.2 M sodium formate and 100 mM Tris buffer, pH=8.0, and kept at 4°C. Crystal showers may appear after 1-2 days with large single crystals growing to full size (0.3 X 0.3 X 0.15 mm<sup>3</sup>) within 2-3 weeks. Crystals are harvested in 3.5 M sodium formate and 100 mM Tris buffer, pH=8.0 and cryoprotected in 3.5 M sodium formate, 100 mM Tris buffer, pH=8.0, 10% (w/v) sucrose, and 10% (v/v) ethylene glycol before flash freezing in liquid propane. In some embodiments, the crystal may be obtained using the methods described in U.S. Patent No. 5,869,604. The method involves (a) contacting a mixture containing uncrystallized polypeptides with an exogenous nucleating agent that has an areal lattice match of at least 90.4% to the polypeptide, (b) crystallizing the polypeptides, thereby forming at least one crystal of the polypeptide attached to the nucleating agent, the attached crystal being of a high purity, and at least one polypeptide crystal unattached to the nucleating agent, the unattached crystal being of a lower purity than the attached crystal, and (c) separating the crystal attached to the nucleating agent from the crystal unattached to the nucleating agent. The crystallized polypeptide may also be purified from contaminants by (a) contacting a mixture containing uncrystallized polypeptides and a contaminant with an exogenous nucleating agent that has an areal lattice match of at least 90.4% to the polypeptide, (b) crystallizing the polypeptides, thereby forming at least one crystal of the polypeptide attached to the nucleating agent, the attached crystal being of a high purity and produced in a high yield, and at least one crystal unattached to the nucleating agent, the unattached crystal being of a lower purity than the attached crystal, and (c) separating the crystal attached to the nucleating agent from the crystal unattached to the nucleating agent.

Once a crystal of the present invention is grown, X-ray diffraction data can be collected using methods familiar to those skilled in the art. Therefore, any person with skill in the art of protein crystallization having the present teachings and without undue experimentation can crystallize a large number of alternative forms of the essential gene products from a variety of different organisms, or polypeptides having conservative substitutions in their amino acid sequence.

A crystal lattice is defined by the symmetry of its unit cell and any structural motifs the unit cell contains. For example, there are 230 possible symmetry groups for an arbitrary crystal lattice, while the unit cell of the crystal lattice group may have an arbitrary dimension that depends on the molecules making up the lattice. Biological macromolecules, however, have

asymmetric centers and are limited to 65 of the 230 symmetry groups. See Cantor et al., Biophysical Chemistry, Vol. III, W. H. Freeman & Company (1980).

5 A crystal lattice interacts with electromagnetic or particle waves, such as X-rays or electron beams respectively, that have a wavelength with the same order of magnitude as the spacing between atoms in the unit cell. The diffracted waves are measured as an array of spots on a detection surface positioned adjacent to the crystal. Each spot has a three-dimensional position,  $hkl$ , and an intensity,  $I(hkl)$ , both of which are used to reconstruct the three-dimensional electron density of the crystal with the so-called Electron Density Equation. The Electron Density Equation states that the three-dimensional electron density of the unit cell is  
10 the Fourier transform of the structure factors. Thus, in theory, if the structure factors are known for a sufficient number of spots in the detection space, then the three-dimensional electron density of the unit cell could be calculated using the Electron Density Equation.

In some embodiments of the present invention, an image of a crystal of a gene product required for proliferation or a portion thereof is obtained with the aid of a digital computer and  
15 the crystal's diffraction pattern as described in U.S. Patent No. 5,353,236. The diffraction pattern contains a plurality of reflections, each having an associated resolution. The image is obtained by (a) converting the diffraction pattern of the crystal into computer usable normalized amplitudes, the pattern being produced with a diffractometer; (b) determining from the diffraction pattern a dimension of a unit cell of the crystal; (c) providing an envelope defining  
20 the region of the unit cell occupied by the gene product or portion thereof in the crystal; (d) distributing a collection of scattering bodies within said envelope, the collection of scattering bodies having various arrangements, each of which has an associated pattern of Fourier amplitudes; (e) condensing the collection of scattering bodies to a condensed arrangement that results in a high correlation between a diffraction pattern and the pattern of Fourier amplitudes  
25 for said collection of scattering bodies; (f) determining the phase associated with at least one of the reflections of said diffraction pattern from the condensed arrangement of scattering bodies; (g) calculating an electron density distribution of the gene product or portion thereof within the unit cell from the phase determined in procedure f; and (h) displaying a graphical image of the gene product or portion thereof constructed from said electron density distribution.

30 The crystals of the gene products required for proliferation may be used in drug screening methods such as those described in U.S. Patent Number 6,156,526. Briefly, in such methods, a compound which inhibits the formation of a complex comprising the gene product or a portion thereof is identified as follows. A set of atomic coordinates defining the three-dimensional structure of a complex including the gene product of interest or a portion thereof  
35 are determined. A potential compound that binds to the gene product or a portion thereof

involved in complex formation is selected using the atomic coordinates obtained above. The compound is contacted with the gene product or portion thereof and its binding partner(s) in the complex under conditions which would permit the complex to form in the absence of the potential compound. The binding affinity of the gene product or portion thereof for its binding partner(s) is determined and a potential compound is identified as a compound that inhibits the formation of the complex when there is a decrease in the binding affinity of the gene product or portion thereof for its binding partner(s).

In some embodiments of the present invention, the three dimensional structure of the essential gene product is determined and potential agonists and/or potential antagonists are designed with the aid of computer modeling [Bugg et al., Scientific American, Dec.:92-98 (1993); West et al., TIPS, 16:67-74 (1995); Dunbrack et al., Folding & Design, 2:27-42 (1997)].

Computer analysis may be performed with one or more of the computer programs including: QUANTA, CHARMM, INSIGHT, SYBYL, MACROMODEL and ICM [Dunbrack et al., Folding & Design, 2:27-42 (1997)]. In a further embodiment of this aspect of the invention, an initial drug-screening assay is performed using the three-dimensional structure so obtained, preferably along with a docking computer program. Such computer modeling can be performed with one or more Docking programs such as FlexX, DOC, GRAM and AUTO DOCK [Dunbrack et al., Folding & Design, 2:27-42 (1997)].

It should be understood that for each drug screening assay provided herein, a number of iterative cycles of any or all of the steps may be performed to optimize the selection. The drug screening assays of the present invention may use any of a number of means for determining the interaction between an agent or drug and an essential gene product.

In some embodiments of the present invention, a drug can be specifically designed to bind to an essential gene product of the present invention through NMR based methodology. [Shuker et al., *sci* Science 274:1531-1534 (1996)]. NMR spectra may be recorded using devices familiar to those skilled in the art, such as the Varian Unity Plus 500 and unity 600 spectrometers, each equipped with a pulsed-field gradient triple resonance probe as analyzed as described in Bagby et al., [Cell 82:857-867 (1995)]. Sequential resonance assignments of backbone  $^1\text{H}$ ,  $^{15}\text{N}$ , and  $^{13}\text{C}$  atoms may be made using a combination of triple resonance experiments similar to those previously described [Bagby et al., Biochemistry, 33:2409-2421 (1994a), except with enhanced sensitivity [Muhandiram and Kay, J. Magn. Reson., 103: 203-216 (1994), and minimal  $\text{H}_2\text{O}$  saturation [Kay et al., J. Magn. Reson., 109:129-133 (1994)]. Side chain  $^1\text{H}$  and  $^{13}\text{C}$  assignments may be made using HCCH-TOCSY [Bax et al., J. Magn. Reson., 87:620-627 (1990)] experiments with mixing times of 8 ms and 16 ms in solution but need not be included in structure calculations. Nuclear Overhauser effect (NOE) cross peaks in

two-dimensional  $^1\text{H}$ -- $^1\text{H}$  NOE spectroscopy (NOESY), three-dimensional  $^{15}\text{N}$ -edited NOESY-HSQC [Zhang et al., J. Biomol. NMR, 4:845-858 (1994)] and three-dimensional simultaneous acquisition  $^{15}\text{N}/^{13}\text{C}$ -edited NOE [Pascal et al., J. Magn. Reson., 103:197-201 (1994)], spectra may be obtained with 100 ms NOE mixing times. Standard pseudo-atom distance corrections  
5 [Wuthrich et al., J. Mol. Biol., 169:949-961 (1983)], may be incorporated to account for center averaging. An additional 0.5 Å. may be added to the upper limits for distances involving methyl groups [Wagner et al., J. Mol. Biol., 196:611-639 (1987); Clore et al., Biochemistry, 26:8012-8023 (1987)].

The structures can be calculated using a simulated annealing protocol [Nilges et al., In  
10 computational Aspects of the Study of Biological Macromolecules by Nuclear Magnetic Resonance Spectroscopy, J. C. Hoch, F. M. Poulsen, and C. Redfield, eds., New York: Plenum Press, pp. 451-455 (1991)], within X-PLOR [Brunger, X-PLOR Manual, Version 3.1, New Haven, Conn.: Department of Molecular Biophysics and Biochemistry, Yale University (1993)], using the previously described strategy [Bagby et al., Structure, 2:107-122 (1994b)].  
15 Interhelical angles may be calculated using a program written by K. Yap. Accessible surface areas were calculated using the program Naccess, available from Prof. J. Thornton, University College, London.

Compounds capable of reducing the activity or amount of gene products required for cellular proliferation may be identified using the methods described in US Pat. No. 6,077,682.  
20 Briefly, the three-dimensional structure of the gene product or portion thereof may be used in a drug screening assay by (a) selecting a potential drug by performing rational drug design with the three-dimensional structure determined from one or more sets of atomic coordinates of the gene product or portion thereof in conjunction with computer modeling; (b) contacting the potential drug with a polypeptide comprising the gene product or portion thereof and (c)  
25 detecting the binding of the potential drug with said polypeptide; wherein a potential drug is selected as a drug if the potential drug binds to the polypeptide. In some methods, the three-dimensional structure of the gene product or portion thereof is used in a drug screening assay involving (a) selecting a potential drug by performing structural based rotational drug design with the three-dimensional structure of the gene product or portion thereof; wherein said  
30 selecting is performed in conjunction with computer modeling; (b) contacting the potential drug with a polypeptide comprising the gene product or portion thereof in the presence of a substrate of the gene product; wherein in the absence of the potential drug the substrate is acted upon by the gene product; and (c) determining the extent to which the gene product acted upon the substrate; wherein a drug is selected when a decrease in the action of the gene product on the  
35 substrate is determined in the presence of the potential drug relative to in its absence. In some

embodiments, the preceding method further involves(d) contacting the potential drug with the gene product or portion thereof for NMR analysis; wherein a binding complex forms between the potential drug and said gene product or portion thereof for NMR analysis; wherein the gene product or portion thereof for NMR analysis comprises a conservative amino acid substitution; (e) determining the three-dimensional structure of the binding complex by NMR; and (f) selecting a candidate drug by performing structural based rational drug design with the three-dimensional structure determined for the binding complex; wherein said selecting is performed in conjunction with computer modeling; (g) contacting the candidate drug with a second polypeptide comprising the gene product or portion thereof in the presence of a substrate of the gene product or portion thereof; wherein in the absence of the candidate drug the substrate is acted upon by the second polypeptide; and (h) determining the amount of action of the second polypeptide on the substrate; wherein a drug is selected when a decrease in the amount of action of the second polypeptide is determined in the presence of the candidate drug relative to in its absence.

Once the three-dimensional structure of a crystal comprising an essential gene product is determined, a potential modulator of its activity, can be examined through the use of computer modeling using a docking program such as FlexX, GRAM, DOCK, or AUTODOCK [Dunbrack et al., 1997, *supra*], to identify potential modulators. This procedure can include computer fitting of potential modulators to the polypeptide or fragments thereof to ascertain how well the shape and the chemical structure of the potential modulator will bind. Computer programs can also be employed to estimate the attraction, repulsion, and steric hindrance of the two binding partners (e.g., the essential gene product and a potential modulator). Generally the tighter the fit, the lower the steric hindrances, and the greater the attractive forces, the more potent the potential modulator since these properties are consistent with a tighter binding constant. Furthermore, the more specificity in the design of a potential drug the more likely that the drug will not interact as well with other proteins. This will minimize potential side-effects due to unwanted interactions with other proteins.

Compound and compound analogs can be systematically modified by computer modeling programs until one or more promising potential analogs is identified. In addition systematic modification of selected analogs can then be systematically modified by computer modeling programs until one or more potential analogs are identified. Such analysis has been shown to be effective in the development of HIV protease inhibitors [Lam et al., *Science* 263:380-384 (1994); Wlodawer et al., *Ann. Rev. Biochem.* 62:543-585 (1993); Appelt, *Perspectives in Drug Discovery and Design* 1:23-48 (1993); Erickson, *Perspectives in Drug Discovery and Design* 1:109-128 (1993)]. Alternatively a potential modulator could be

obtained by initially screening a random peptide library produced by recombinant bacteriophage for example, [Scott and Smith, Science, 249:386-390 (1990); Cwirla et al., Proc. Natl. Acad. Sci., 87:6378-6382 (1990); Devlin et al., Science, 249:404-406 (1990)]. A peptide selected in this manner would then be systematically modified by computer modeling programs as described above, and then treated analogously to a structural analog.

Example 91 describes computer modelling of the structures of gene products required for proliferation.

#### EXAMPLE 91

##### Determination of the Structure of Gene Products Required for Proliferation Using Computer

##### Modelling

Three dimensional models were built by applying computer modelling methods to some of the gene products required for proliferation of *Staphylococcus aureus* using the amino acid sequences of the encoded proteins as follows. Sir Tom Blundell's program COMPOSER as provided by Tripos Associates in their BIOPOLYMER module to SYBYL was used to build the models. Skolnik's method of topology fingerprinting as implemented in Matchmaker was used to score the average mutation free energy. This number is in Boltzmanns (units of kT) and should be negative (the more negative, the better the model).

Composer uses a Needleman Wunsch alignment with jumbling to find significant alignments. The reported parameters are percent identity and significance as measured from the jumbling. Those matches which were 30% identical and had a significance greater than 4 on the scale were judged to be good candidates for model building templates. If no three dimensional structures met these criteria, then a BLAST search was conducted against the most recent PDB sequence database. Any significant hits discovered in this manner were then added to the binary protein structure database and the candidate search was repeated in the manner discussed above.

In the next phase, Composer assigned structurally conserved and structurally variable regions and built the backbone structure and then searched the database for structures of the variable loops. These were then spliced in and a model of the protein resulted. Any loops (variable regions) which were unassignable were manually built and refined with a combination of dynamics.

The structure was then refined. Hydrogen atoms were added and a non-active aggregate was defined. 1000ps of dynamics using AMBER ALL-ATOM and Kollman charges are performed. Next a minimization cycle of up 5000 steepest decent steps were performed and then the aggregate was thawed and the process was repeated on the entire protein.

The resulting structure was then validated in MATCHMAKER. The topologically scanned free energy determined from empirically derived protein topologies was computed and the average energy/residue is reported in Boltzmann was reported. As this number represents a free energy the more negative it is the more favorable it is.

5           Sixty six proteins required for the proliferation of *Staphylococcus aureus* were modelled as described above. MATCHMAKER energies were computed for these. The distribution of the models built by class is shown in Table VIII below.

Table VIII: Distribution of models built with their MATCHMAKER energies in kT

Classification	Number of Models	Average Matchmaker Energy
Acylases	1	-0.10
Dehydrogenases	3	-0.12
DNA Related	3	-0.12
Heat Shock Protein	2	-0.16
Hydrolases	3	-0.16
Isomerases	1	0.05
Ligases	7	-0.07
Lyases	1	-0.09
Membrane Anchored	1	-0.12
Misc	18	-0.21
Oxidoreductases	6	-0.09
Proteases	1	-0.03
Ribosome	3	-0.11
Synthases	4	-0.14
Transferases	6	-0.12

10           The validity of the above method was confirmed using FtsZ. In the case of FtsZ, a crystal structure from M. Janeschi was available. Examination of the gross structural features determined using the above modelling showed all of the folds in the correct place, although there were some minor differences from the structure determined by x-ray crystallography.

#### EXAMPLE 92

##### Functional Complementation

15           In another embodiment, gene products whose activities may be complemented by a proliferation-required gene product from *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Enterobacter cloacae*, *Enterococcus faecium*, *Haemophilus influenzae*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*,

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*Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Proteus mirabilis*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*,  
5 *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae* or *Yersinia pestis* or homologous polypeptides are identified using merodiploids, created by introducing a plasmid or Bacterial Artificial Chromosome into an organism having a mutation in the essential gene which reduces or eliminates the activity of the gene product. In some embodiments, the mutation may be a conditional mutation, such as a temperature sensitive mutation, such that the  
10 organism proliferates under permissive conditions but is unable to proliferate under non-permissive conditions in the absence of complementation by the gene on the plasmid or Bacterial Artificial Chromosome. Alternatively, duplications may be constructed as described in Roth et al. (1987) Biosynthesis of Aromatic Amino Acids in *Escherichia coli* and *Salmonella typhimurium*, F. C. *Neidhardt*, ed., American Society for Microbiology, publisher, pp. 2269-  
15 2270. Such methods are familiar to those skilled in the art.

It will be appreciated that no matter how detailed the foregoing appears in text, the invention can be practiced in many ways. As is also stated above, it should further be noted that the use of particular terminology when describing certain features or aspects of the present invention should not be taken to imply that the broadest reasonable meaning of such terminology is  
20 not intended, or that the terminology is being re-defined herein to be restricted to including any specific characteristics of the features or aspects of the invention with which that terminology is associated. Thus, although this invention has been described in terms of certain preferred embodiments, other embodiments which will be apparent to those of ordinary skill in the art in view of the disclosure herein are also within the scope of this invention. Accordingly, the scope of  
25 the invention is intended to be defined only by reference to the appended claims and any equivalents thereof.



WHAT IS CLAIMED IS:

1. A purified or isolated nucleic acid sequence comprising a nucleotide sequence consisting essentially of one of SEQ ID NOs: 1-6213, wherein expression of said nucleic acid inhibits proliferation of a cell.

5 2. The nucleic acid sequence of Claim 1, wherein said nucleotide sequence is complementary to at least a portion of a coding sequence of a gene whose expression is required for proliferation of a cell.

3. The nucleic acid of Claim 1, wherein said nucleic acid sequence is complementary to at least a portion of a nucleotide sequence of an RNA required for  
10 proliferation of a cell.

4. The nucleic acid of Claim 3, wherein said RNA is an RNA comprising a sequence of nucleotides encoding more than one gene product.

5. A purified or isolated nucleic acid comprising a fragment of one of SEQ ID NOs: 1-6213, said fragment selected from the group consisting of fragments comprising at least  
15 10, at least 20, at least 25, at least 30, at least 50 and more than 50 consecutive nucleotides of one of SEQ ID NOs: 1-6213.

6. The fragment of Claim 5, wherein said fragment is included in a nucleic acid obtained from an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*,  
20 *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium*  
25 *botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*,  
30 *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*,  
35 *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella*

*sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

7. The fragment of Claim 5, wherein said fragment is included in a nucleic acid obtained from an organism other than *Escherichia coli*.

8. A vector comprising a promoter operably linked to the nucleic acid of any one of Claims 1-7.

9. The vector of Claim 8, wherein said promoter is active in a microorganism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefir* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

10. A host cell containing the vector of Claim 8 or Claim 9.

11. A purified or isolated antisense nucleic acid comprising a nucleotide sequence complementary to at least a portion of an intragenic sequence, intergenic sequence, sequences spanning at least a portion of two or more genes, 5' noncoding region, or 3' noncoding region within an operon comprising a proliferation-required gene whose activity or expression is inhibited by an antisense nucleic acid comprising the nucleotide sequence of one of SEQ ID NOs: 1-6213.

12. The purified or isolated antisense nucleic acid of Claim 11, wherein said antisense nucleic acid is complementary to a nucleic acid from an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

13. The purified or isolated antisense nucleic acid of Claim 11, wherein said nucleotide sequence is complementary to a nucleotide sequence of a nucleic acid from an organism other than *E. coli*.

14. The purified or isolated antisense nucleic acid of Claim 11, wherein said proliferation-required gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

15. A purified or isolated nucleic acid comprising a nucleotide sequence having at least 70% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213, fragments comprising at least 25 consecutive nucleotides of SEQ ID NOS.: 1-6213, the nucleotide sequences complementary to SEQ ID NOS.: 1-6213 and the sequences complementary to fragments comprising at least 25 consecutive nucleotides of SEQ ID NOS.: 1-6213 as determined using BLASTN version 2.0 with the default parameters.

16. The purified or isolated nucleic acid of Claim 15, wherein said nucleic acid is obtained from an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

17. The nucleic acid of Claim 15, wherein said nucleic acid is obtained from an organism other than *E. coli*.

18. A vector comprising a promoter operably linked to a nucleic acid encoding a polypeptide whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence of any one of SEQ ID NOs.: 1-6213.

19. The vector of Claim 18, wherein said nucleic acid encoding said polypeptide is  
5 obtained from an organism selected from the group consisting of *Acinetobacter baumannii*,  
*Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*,  
*Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*,  
*Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called  
*Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*,  
10 *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*,  
*Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium*  
*botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*,  
*Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus*  
*faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*,  
15 *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria*  
*monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*,  
*Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma*  
*pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella*  
*haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*,  
20 *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*,  
*Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*,  
*Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella*  
*sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*,  
*Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema*  
25 *pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio*  
*vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of  
any of the above species.

20. The vector of Claim 18, wherein said nucleotide sequence encoding said polypeptide is obtained from an organism other than *E. coli*.

30 21. A host cell containing the vector of Claim 18.

22. The vector of Claim 18, wherein said polypeptide comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 42398-78581.

23. The vector of Claim 18, wherein said promoter is operably linked to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

24. A purified or isolated polypeptide comprising a polypeptide whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence of any one of SEQ ID NOS.: 1-6213, or a fragment selected from the group consisting of fragments comprising at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60 or more than 60 consecutive amino acids of one of the said polypeptides.

25. The polypeptide of Claim 24, wherein said polypeptide comprises an amino acid sequence of any one of SEQ ID NOS.: 42398-78581 or a fragment comprising at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60 or more than 60 consecutive amino acids of a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS.: 42398-78581.

26. The polypeptide of Claim 24, wherein said polypeptide is obtained from an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio*

*vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

27. The polypeptide of Claim 24, wherein said polypeptide is obtained from an organism other than *E. coli*.

5 28. A purified or isolated polypeptide comprising a polypeptide having at least 25% amino acid identity to a polypeptide whose expression is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, or at least 25% amino acid identity to a fragment comprising at least 10, at least 20, at least 30, at least 40, at least 50, at least 60 or more than 60 consecutive amino acids of a polypeptide  
10 whose expression is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 as determined using FASTA version 3.0t78 with the default parameters.

29. The polypeptide of Claim 28, wherein said polypeptide has at least 25% identity to a polypeptide comprising one of SEQ ID NOs: 42398-78581 or at least 25% identity  
15 to a fragment comprising at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60 or more than 60 consecutive amino acids of a polypeptide comprising one of SEQ ID NOs.: 42398-78581 as determined using FASTA version 3.0t78 with the default parameters.

30. The polypeptide of Claim 28, wherein said polypeptide is obtained from an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*,  
20 *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*,  
25 *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*,  
30 *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*,  
35 *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella*

boydii, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

31. The polypeptide of Claim 28, wherein said polypeptide is obtained from an organism other than *E. coli*.

32. An antibody capable of specifically binding the polypeptide of one of Claims 28-31.

33. A method of producing a polypeptide, comprising introducing a vector comprising a promoter operably linked to a nucleic acid comprising a nucleotide sequence encoding a polypeptide whose expression is inhibited by an antisense nucleic acid comprising one of SEQ ID NOs.: 1-6213 into a cell.

34. The method of Claim 33, further comprising the step of isolating said polypeptide.

35. The method of Claim 33, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

36. The method of Claim 33, wherein said nucleic acid encoding said polypeptide is obtained from an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*,



5 *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

37. The method of Claim 33, wherein said nucleic acid encoding said polypeptide is obtained from an organism other than *E. coli*.

10 38. The method of Claim 33, wherein said promoter is operably linked to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

15 39. A method of inhibiting proliferation of a cell in an individual comprising inhibiting the activity or reducing the amount of a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213 or inhibiting the activity or reducing the amount of a nucleic acid encoding said gene product.

20 40. The method of Claim 39, wherein said method comprises inhibiting said activity or reducing said amount of a gene product in an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*,  
25 *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*,  
30 *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*,  
35 *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella*

*boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

41. The method of Claim 39, wherein said method comprises inhibiting said activity or reducing said amount of a gene product in an organism other than *E. coli*.

42. The method of Claim 39, wherein said gene product is present in an organism other than *E. coli*.

43. The method of Claim 39, wherein said gene product comprises a polypeptide comprising a sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

44. A method for identifying a compound which influences the activity of a gene product required for proliferation, said gene product comprising a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, said method comprising:

contacting said gene product with a candidate compound; and

determining whether said compound influences the activity of said gene product.

45. The method of Claim 44, wherein said gene product is from an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*,

Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

46. The method of Claim 44, wherein said gene product is from an organism other than *E. coli*.

47. The method of Claim 44, wherein said gene product is a polypeptide and said activity is an enzymatic activity.

48. The method of Claim 44, wherein said gene product is a polypeptide and said activity is a carbon compound catabolism activity.

49. The method of Claim 44, wherein said gene product is a polypeptide and said activity is a biosynthetic activity.

50. The method of Claim 44, wherein said gene product is a polypeptide and said activity is a transporter activity.

51. The method of Claim 44, wherein said gene product is a polypeptide and said activity is a transcriptional activity.

52. The method of Claim 44, wherein said gene product is a polypeptide and said activity is a DNA replication activity.

53. The method of Claim 44, wherein said gene product is a polypeptide and said activity is a cell division activity.

54. The method of Claim 44, wherein said gene product is an RNA.

55. The method of Claim 44, wherein said gene product is a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

56. A compound identified using the method of Claim 44.

57. A method for identifying a compound or nucleic acid having the ability to reduce the activity or level of a gene product required for proliferation, said gene product comprising a gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, said method comprising:

(a) contacting a target gene or RNA encoding said gene product with a candidate compound or nucleic acid; and

(b) measuring an activity of said target.

58. The method of Claim 57, wherein said target gene or RNA is from an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*,  
5 *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*,  
10 *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*,  
20 *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

59. The method of Claim 57, wherein said target gene or RNA is from an organism  
25 other than *E. coli*.

60. The method of Claim 57, wherein said gene product is from an organism other than *E. coli*.

61. The method of Claim 57, wherein said target is a messenger RNA molecule and said activity is translation of said messenger RNA.

62. The method of Claim 57, wherein said target is a messenger RNA molecule and said activity is transcription of a gene encoding said messenger RNA.  
30

63. The method of Claim 57, wherein said target is a gene and said activity is transcription of said gene.

64. The method of Claim 57, wherein said target is a nontranslated RNA and said activity is processing or folding of said nontranslated RNA or assembly of said nontranslated RNA into a protein/RNA complex.

65. The method of Claim 57, wherein said target is a messenger RNA molecule encoding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS.: 42398-78581.

66. The method of Claim 57, wherein said target comprises a nucleic acid selected from the group consisting of SEQ ID NOS.: 6214-42397.

67. A compound or nucleic acid identified using the method of Claim 57.

68. A method for identifying a compound which reduces the activity or level of a gene product required for proliferation of a cell, wherein the activity or expression of said gene product is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213, said method comprising the steps of:

(a) providing a sublethal level of an antisense nucleic acid comprising a nucleotide sequence complementary to a nucleic acid comprising a nucleotide sequence encoding said gene product in a cell to reduce the activity or amount of said gene product in said cell, thereby producing a sensitized cell;

(b) contacting said sensitized cell with a compound; and

(c) determining the degree to which said compound inhibits proliferation of said sensitized cell relative to a cell which does not contain said antisense nucleic acid.

69. The method of Claim 68, wherein said determining step comprises determining whether said compound inhibits the growth of said sensitized cell to a greater extent than said compound inhibits the growth of a nonsensitized cell.

70. The method of Claim 68, wherein said cell is a Gram positive bacterium.

71. The method of Claim 68, wherein said Gram positive bacterium is selected from the group consisting of *Staphylococcus* species, *Streptococcus* species, *Enterococcus* species, *Mycobacterium* species, *Clostridium* species, and *Bacillus* species.

72. The method of Claim 68, wherein said bacterium is *Staphylococcus aureus*.

73. The method of Claim 72, wherein said *Staphylococcus* species is coagulase negative.

74. The method of Claim 72, wherein said bacterium is selected from the group consisting of *Staphylococcus aureus* RN450 and *Staphylococcus aureus* RN4220.

75. The method of Claim 68, wherein said cell is an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia*

cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans,  
 Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis,  
 Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis),  
 Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium  
 5 acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,  
 Coccidioides immitis, Corynebacterium diphtheriae, Cryptococcus neoformans, Enterobacter  
 cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus  
 influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella  
 pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,  
 10 Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma  
 genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia  
 asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus  
 mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas  
 syringae, Salmonella bongori, Salmonella choleraesuis, Salmonella enterica, Salmonella  
 15 paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae,  
 Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis,  
 Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus  
 pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio  
 parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species  
 20 falling within the genera of any of the above species.

76. The method of Claim 68, wherein said cell is not an *E. coli* cell.

77. The method of Claim 68, wherein said gene product is from an organism other than *E. coli*.

78. The method of Claim 68, wherein said antisense nucleic acid is transcribed  
 25 from an inducible promoter.

79. The method of Claim 68, further comprising the step of contacting said cell with a concentration of inducer which induces transcription of said antisense nucleic acid to a sublethal level.

80. The method of Claim 68, wherein growth inhibition is measured by monitoring  
 30 optical density of a culture growth solution.

81. The method of Claim 68, wherein said gene product is a polypeptide.

82. The method of Claim 81, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

83. The method of Claim 68, wherein said gene product is an RNA.

84. The method of Claim 68, wherein nucleic acid encoding said gene product comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

85. A compound identified using the method of Claim 68.

5 86. A method for inhibiting cellular proliferation comprising introducing an effective amount of a compound with activity against a gene whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213 or a compound with activity against the product of said gene into a population of cells expressing said gene.

10 87. The method of Claim 86, wherein said compound is an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213, or a proliferation-inhibiting portion thereof.

88. The method of Claim 86, wherein said proliferation inhibiting portion of one of SEQ ID NOS.: 1-6213 is a fragment comprising at least 10, at least 20, at least 25, at least 30, at  
15 least 50 or more than 51 consecutive nucleotides of one of SEQ ID NOS.: 1-6213.

89. The method of Claim 86, wherein said population is a population of Gram positive bacteria.

90. The method of Claim 89, wherein said population of Gram positive bacteria is selected from the group consisting of a population of *Staphylococcus* species, *Streptococcus*  
20 species, *Enterococcus* species, *Mycobacterium* species, *Clostridium* species, and *Bacillus* species.

91. The method of Claim 86, wherein said population is a population of *Staphylococcus aureus*.

92. The method of Claim 91, wherein said population is a population of a  
25 bacterium selected from the group consisting of *Staphylococcus aureus* RN450 and *Staphylococcus aureus* RN4220.

93. The method of Claim 86, wherein said population is a population of a  
bacterium selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*,  
30 *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*,  
35 *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*,

Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella choleraesuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

94. The method of Claim 86, wherein said population is a population of an organism other than *E. coli*.

95. The method of Claim 86, wherein said product of said gene is from an organism other than *E. coli*.

96. The method of Claim 86, wherein said gene encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS.: 42398-78581.

97. The method of Claim 86, wherein said gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

98. A composition comprising an effective concentration of an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213, or a proliferation-inhibiting portion thereof in a pharmaceutically acceptable carrier.

99. The composition of Claim 98, wherein said proliferation-inhibiting portion of one of SEQ ID NOS.: 1-6213 comprises at least 20, at least 25, at least 30, at least 50 or more than 50 consecutive nucleotides of one of SEQ ID NOS.: 1-6213.

100. A method for inhibiting the activity or expression of a gene in an operon required for proliferation wherein the activity or expression of at least one gene in said operon is inhibited by an antisense nucleic acid comprising a sequence selected from the group consisting of SEQ ID NOS.: 1-6213, said method comprising contacting a cell in a cell population with an antisense nucleic acid complementary to at least a portion of said operon.



101. The method of Claim 100, wherein said antisense nucleic acid comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 or a proliferation-inhibiting portion thereof.

102. The method of Claim 100, wherein said cell is selected from the group  
5 consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*,  
10 *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*,  
15 *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*,  
20 *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of  
25 any of the above species.

103. The method of Claim 100, wherein said cell is not an *E. coli* cell.

104. The method of Claim 100, wherein said gene is from an organism other than *E. coli*.

105. The method of Claim 100, wherein said cell is contacted with said antisense nucleic acid by introducing a plasmid which expresses said antisense nucleic acid into said cell  
30 population.

106. The method of Claim 100, wherein said cell is contacted with said antisense nucleic acid by introducing a phage which encodes said antisense nucleic acid into said cell  
35 population.

107. The method of Claim 100, wherein said cell is contacted with said antisense nucleic acid by expressing said antisense nucleic acid from the chromosome of cells in said cell population.

108. The method of Claim 100, wherein said cell is contacted with said antisense nucleic acid by introducing a promoter adjacent to a chromosomal copy of said antisense nucleic acid such that said promoter directs the transcription of said antisense nucleic acid.

109. The method of Claim 100, wherein said cell is contacted with said antisense nucleic acid by introducing a retron which expresses said antisense nucleic acid into said cell population.

110. The method of Claim 100, wherein said cell is contacted with said antisense nucleic acid by introducing a ribozyme into said cell-population, wherein a binding portion of said ribozyme comprises said antisense nucleic acid.

111. The method of Claim 100, wherein said cell is contacted with said antisense nucleic acid by introducing a liposome comprising said antisense nucleic acid into said cell.

112. The method of Claim 100, wherein said cell is contacted with said antisense nucleic acid by electroporation of said antisense nucleic acid into said cell.

113. The method of Claim 100, wherein said antisense nucleic acid is a fragment comprising at least 10, at least 20, at least 25, at least 30, at least 50 or more than 50 consecutive nucleotides of one of SEQ ID NOs.: 1-6213.

114. The method of Claim 100 wherein said antisense nucleic acid is a synthetic oligonucleotide.

115. The method of Claim 100, wherein said gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

116. A method for identifying a gene which is required for proliferation of a cell comprising:

(a) contacting a cell with an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, wherein said cell is a cell other than the organism from which said nucleic acid was obtained;

(b) determining whether said nucleic acid inhibits proliferation of said cell; and

(c) identifying the gene in said cell which encodes the mRNA which is complementary to said antisense nucleic acid or a portion thereof.

117. The method of Claim 116, wherein said cell is selected from the group consisting of *Staphylococcus* species, *Streptococcus* species, *Enterococcus* species, *Mycobacterium* species, *Clostridium* species, and *Bacillus* species.

118. The method of Claim 116 wherein said cell is selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

119. The method of Claim 116, wherein said cell is not *E. coli*.

120. The method of Claim 116, further comprising operably linking said antisense nucleic acid to a promoter which is functional in said cell, said promoter being included in a vector, and introducing said vector into said cell.

121. A method for identifying a compound having the ability to inhibit proliferation of a cell comprising:

- (a) identifying a homolog of a gene or gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs. 1-6213 in a test cell, wherein said test cell is not the cell from which said nucleic acid was obtained;
- (b) identifying an inhibitory nucleic acid sequence which inhibits the activity of said homolog in said test cell;

(c) contacting said test cell with a sublethal level of said inhibitory nucleic acid, thus sensitizing said cell;

(d) contacting the sensitized cell of step (c) with a compound; and

(e) determining the degree to which said compound inhibits proliferation of  
5 said sensitized cell relative to a cell which does not contain said inhibitory nucleic acid.

122. The method of Claim 121, wherein said determining step comprises determining whether said compound inhibits proliferation of said sensitized test cell to a greater extent than said compound inhibits proliferation of a nonsensitized test cell.

123. The method of Claim 121, wherein step (a) comprises identifying a nucleic acid  
10 homologous to a gene or gene product whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213 or a nucleic acid encoding a homologous polypeptide to a polypeptide whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213 by using an algorithm selected from the group consisting of BLASTN version 2.0 with the default parameters and FASTA version  
15 3.0t78 algorithm with the default parameters to identify said homologous nucleic acid or said nucleic acid encoding a homologous polypeptide in a database.

124. The method of Claim 121 wherein said step (a) comprises identifying a homologous nucleic acid or a nucleic acid comprising a sequence of nucleotides encoding a homologous polypeptide by identifying nucleic acids which hybridize to said nucleic acid  
20 selected from the group consisting of SEQ ID NOs. 1-6213 or the complement of said nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213.

125. The method of Claim 121 wherein step (a) comprises expressing a nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213 in said test cell.

126. The method of Claim 121, wherein step (a) comprises identifying a  
25 homologous nucleic acid or a nucleic acid encoding a homologous polypeptide in a test cell selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*),  
30 *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*,  
35 *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*,

*Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*,  
 5 *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*,  
 10 *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

127. The method of Claim 121, wherein step (a) comprises identifying a  
 15 homologous nucleic acid or a nucleic acid encoding a homologous polypeptide in a test cell other than *E. coli*.

128. The method of Claim 121, wherein said inhibitory nucleic acid is an antisense nucleic acid.

129. The method of Claim 121, wherein said inhibitory nucleic acid comprises an  
 20 antisense nucleic acid to a portion of said homolog.

130. The method of Claim 121, wherein said inhibitory nucleic acid comprises an antisense nucleic acid to a portion of the operon encoding said homolog.

131. The method of Claim 121, wherein the step of contacting the cell with a  
 25 sublethal level of said inhibitory nucleic acid comprises directly contacting the surface of said cell with said inhibitory nucleic acid.

132. The method of Claim 121, wherein the step of contacting the cell with a sublethal level of said inhibitory nucleic acid comprises transcribing an antisense nucleic acid complementary to at least a portion of the RNA transcribed from said homolog in said cell.

133. The method of Claim 121, wherein said gene product comprises a polypeptide  
 30 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS.: 42398-78581.

134. The method of Claim 121, wherein said gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

135. A compound identified using the method of Claim 121.

136. A method of identifying a compound having the ability to inhibit proliferation comprising:

(a) contacting a test cell with a sublethal level of a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs. 1-6213 or a portion thereof which inhibits the proliferation of the cell from which said nucleic acid was obtained, thus sensitizing said test cell;

(b) contacting the sensitized test cell of step (a) with a compound; and

(c) determining the degree to which said compound inhibits proliferation of said sensitized test cell relative to a cell which does not contain said nucleic acid.

137. The method of Claim 136, wherein said determining step comprises determining whether said compound inhibits proliferation of said sensitized test cell to a greater extent than said compound inhibits proliferation of a nonsensitized test cell.

138. A compound identified using the method of Claim 136.

139. The method of Claim 136, wherein said test cell is selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio*

vulnificans, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

140. The method of Claim 136, wherein the test cell is not *E. coli*.

141. A method for identifying a compound having activity against a biological pathway required for proliferation comprising:

(a) sensitizing a cell by providing a sublethal level of an antisense nucleic acid complementary to a nucleic acid encoding a gene product required for proliferation, wherein the activity or expression of said gene product is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, in said cell to reduce the activity or amount of said gene product;

(b) contacting the sensitized cell with a compound; and

(c) determining the degree to which said compound inhibits the growth of said sensitized cell relative to a cell which does not contain said antisense nucleic acid.

142. The method of Claim 141, wherein said determining step comprises determining whether said compound inhibits the growth of said sensitized cell to a greater extent than said compound inhibits the growth of a nonsensitized cell.

143. The method of Claim 141, wherein said cell is selected from the group consisting of bacterial cells, fungal cells, plant cells, and animal cells.

144. The method of Claim 141, wherein said cell is a Gram positive bacterium.

145. The method of Claim 144, wherein said Gram positive bacterium is selected from the group consisting of *Staphylococcus* species, *Streptococcus* species, *Enterococcus* species, *Mycobacterium* species, *Clostridium* species, and *Bacillus* species.

146. The method of Claim 145, wherein said Gram positive bacterium is *Staphylococcus aureus*.

147. The method of Claim 146, wherein said Gram positive bacterium is selected from the group consisting of *Staphylococcus aureus* RN450 and *Staphylococcus aureus* RN4220.

148. The method of Claim 141, wherein said cell is selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*,

Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diphtheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella choleraesuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

149. The method of Claim 141, wherein said cell is not an *E. coli* cell.

150. The method of Claim 141, wherein said gene product is from an organism other than *E. coli*.

151. The method of Claim 141, wherein said antisense nucleic acid is transcribed from an inducible promoter.

152. The method of Claim 141, further comprising contacting the cell with an agent which induces transcription of said antisense nucleic acid from said inducible promoter, wherein said antisense nucleic acid is transcribed at a sublethal level.

153. The method of Claim 141, wherein inhibition of proliferation is measured by monitoring the optical density of a liquid culture.

154. The method of Claim 141, wherein said gene product comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

155. The method of Claim 141, wherein said nucleic acid encoding said gene product comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

156. A compound identified using the method of Claim 141.

157. A method for identifying a compound having the ability to inhibit cellular proliferation comprising:



(a) contacting a cell with an agent which reduces the activity or level of a gene product required for proliferation of said cell, wherein said gene product is a gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213;

(b) contacting said cell with a compound; and

(c) determining whether said compound reduces proliferation of said contacted cell by acting on said gene product.

158. The method of Claim 157, wherein said determining step comprises determining whether said compound reduces proliferation of said contacted cell to a greater extent than said compound reduces proliferation of cells which have not been contacted with said agent.

159. The method of Claim 157, wherein said cell is selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

160. The method of Claim 157, wherein said cell is not an *E. coli* cell.

161. The method of Claim 157, wherein said gene product is from an organism other than *E. coli*.

5 162. The method of Claim 157, wherein said agent which reduces the activity or level of a gene product required for proliferation of said cell comprises an antisense nucleic acid to a gene or operon required for proliferation.

163. The method of Claim 157, wherein said agent which reduces the activity or level of a gene product required for proliferation of said cell comprises a compound known to inhibit growth or proliferation of a cell.

10 164. The method of Claim 157, wherein said cell contains a mutation which reduces the activity or level of said gene product required for proliferation of said cell.

165. The method of Claim 157, wherein said mutation is a temperature sensitive mutation.

15 166. The method of Claim 157, wherein said gene product comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

167. A compound identified using the method of Claim 157.

168. A method for identifying the biological pathway in which a proliferation-required gene or its gene product lies, wherein said gene or gene product comprises a gene or gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a sequence selected from the group consisting of SEQ ID NOs.: 1-6213, said method comprising:

- 20 (a) providing a sublethal level of an antisense nucleic acid which inhibits the activity of said proliferation-required gene or gene product in a test cell;
- 25 (b) contacting said test cell with a compound known to inhibit growth or proliferation of a cell, wherein the biological pathway on which said compound acts is known; and
- (c) determining the degree to which said proliferation of said test cell is inhibited relative to a cell which was not contacted with said compound.

30 169. The method of Claim 168, wherein said determining step comprises determining whether said test cell has a substantially greater sensitivity to said compound than a cell which does not express said sublethal level of said antisense nucleic acid.

170. The method of Claim 168, wherein said gene product comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

171. The method of Claim 168, wherein said test cell is selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

172. The method of Claim 168, wherein said test cell is not an *E. coli* cell.

173. The method of Claim 168, wherein said gene product is from an organism other than *E. coli*.

174. A method for determining the biological pathway on which a test compound acts comprising:

(a) providing a sublethal level of an antisense nucleic acid complementary to a proliferation-required nucleic acid in a first cell, wherein the activity or expression of said proliferation-required nucleic acid is inhibited by an antisense nucleic acid comprising a sequence selected from the group consisting of SEQ ID NOs.: 1-6213 and wherein the biological pathway in which said proliferation-required nucleic acid or a protein encoded by said proliferation-required nucleic acid lies is known,

(b) contacting said first cell with said test compound; and

(c) determining the degree to which said test compound inhibits proliferation of said first cell relative to a cell which does not contain said antisense nucleic acid.

175. The method of Claim 174, wherein said determining step comprises determining whether said first cell has a substantially greater sensitivity to said test compound than a cell which does not express said sublethal level of said antisense nucleic acid.

176. The method of Claim 174, further comprising:

(d) providing a sublethal level of a second antisense nucleic acid complementary to a second proliferation-required nucleic acid in a second cell, wherein said second proliferation-required nucleic acid is in a different biological pathway than said proliferation-required nucleic acid in step (a); and

(e) determining whether said second cell does not have a substantially greater sensitivity to said test compound than a cell which does not express said sublethal level of said second antisense nucleic acid, wherein said test compound is specific for the biological pathway against which the antisense nucleic acid of step (a) acts if said first cell has a substantially greater sensitivity to said test compound than said second cell.

177. The method of Claim 174, wherein said first cell is selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema*

pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

178. The method of Claim 174, wherein said first cell is not an *E. coli* cell.

5 179. The method of Claim 174, wherein said proliferation-required nucleic acid is from an organism other than *E. coli*.

180. A purified or isolated nucleic acid comprising a sequence selected from the group consisting of SEQ ID NOs.: 1-6213.

10 181. A compound which interacts with a gene or gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence of one of SEQ ID NOs.: 1-6213 to inhibit proliferation.

182. The compound of Claim 181, wherein said gene product is a polypeptide comprising one of SEQ ID NOs.: 42398-78581.

15 183. The compound of Claim 181, wherein said gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

184. A compound which interacts with a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence of one of SEQ ID NOs.: 1-6213 to inhibit proliferation.

20 185. A method for manufacturing an antibiotic comprising the steps of:  
screening one or more candidate compounds to identify a compound that reduces the activity or level of a gene product required for proliferation, said gene product comprising a gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213; and  
manufacturing the compound so identified.

25 186. The method of Claim 185, wherein said screening step comprises performing any one of the methods of Claims 44, 68, 121, 136, 141, and 157.

187. The method of Claim 185, wherein said gene product is a polypeptide comprising one of SEQ ID NOs.: 42398-78581.

30 188. A method for inhibiting proliferation of a cell in a subject comprising administering an effective amount of a compound that reduces the activity or level of a gene product required for proliferation of said cell, said gene product comprising a gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 to said subject.

35 189. The method of Claim 188 wherein said subject is selected from the group consisting of vertebrates, mammals, avians, and human beings.

190. The method of Claim 188, wherein said gene product comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

191. The method of Claim 188, wherein said cell is selected from the group  
5 consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*,  
10 *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*,  
15 *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*,  
20 *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of  
25 any of the above species.

192. The method of Claim 188, wherein said cell is not *E. coli*.

193. The method of Claim 188, wherein said gene product is from an organism other than *E. coli*.

194. A purified or isolated nucleic acid consisting essentially of the coding sequence  
30 of one of SEQ ID NOs: 6214-42397.

195. A fragment of the nucleic acid of Claim 8, said fragment comprising at least 10, at least 20, at least 25, at least 30, at least 50 or more than 50 consecutive nucleotides of one of SEQ ID NOs: 6214-42397.

196. A purified or isolated nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, fragments comprising at least 25 consecutive nucleotides of SEQ ID NOs.: 6214-42397, the nucleotide sequences complementary to SEQ ID NOs.: 6214-42397, and the nucleotide sequences complementary to fragments comprising at least 25 consecutive nucleotides of SEQ ID NOs.: 6214-42397 as determined using BLASTN version 2.0 with the default parameters.

197. The nucleic acid of Claim 196, wherein said nucleic acid is from an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

198. The nucleic acid of Claim 196, wherein said nucleic acid is from an organism other than *E. coli*.

199. A method of inhibiting proliferation of a cell comprising inhibiting the activity or reducing the amount of a gene product in said cell or inhibiting the activity or reducing the amount of a nucleic acid encoding said gene product in said cell, wherein said gene product is

selected from the group consisting of a gene product having having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213.

200. The method of Claim 199, wherein said method comprises inhibiting said activity or reducing said amount of said gene product or inhibiting the activity or reducing the amount of a nucleic acid encoding said gene product in an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diptheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*,



*Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*,  
 5 *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

201. The method of Claim 199, wherein said method comprises inhibiting said  
 10 activity or reducing said amount of said gene product or inhibiting the activity or reducing the amount of a nucleic acid encoding said gene product in an organism other than *E. coli*.

202. The method of Claim 199, wherein said gene product is from an organism other than *E. coli*.

203. The method of Claim 199, wherein said gene product comprises a polypeptide  
 15 selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42398-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs.: 42398-78581.

204. The method of Claim 199, wherein said gene product is encoded by a nucleic  
 20 acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent  
 25 conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions.

205. A method for identifying a compound which influences the activity of a gene product required for proliferation comprising:

30 contacting a candidate compound with a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product  
 35 encoded by a nucleic acid having at least 70% nucleotide sequence identity as

determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using

5 FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product

10 encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213; and

15 determining whether said candidate compound influences the activity of said gene product.

206. The method of Claim 205, wherein said gene product is from an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*,

25 *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*,

30 *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*,

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*Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

5           207. The method of Claim 205, wherein said gene product is from an organism other than *E. coli*.

          208. The method of Claim 205, wherein said gene product is a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of  
10       SEQ ID NOS.: 42398-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOS: 42398-78581.

          209. The method of Claim 205, wherein said gene product is encoded by a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the  
15       default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions.

20           210. A compound identified using the method of Claim 205.

          211. A method for identifying a compound or nucleic acid having the ability to reduce the activity or level of a gene product required for proliferation comprising:

          (a) providing a target that is a gene or RNA, wherein said target comprises a nucleic acid that encodes a gene product selected from the group consisting of a gene  
25       product having having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleic acid identity as determined using BLASTN version 2.0 with  
30       the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a  
35       sequence selected from the group consisting of SEQ ID NOS.: 1-6213, a gene product

encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213;

(b) contacting said target with a candidate compound or nucleic acid; and

(c) measuring an activity of said target.

212. The method of Claim 211, wherein said target gene or RNA is from an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

213. The method of Claim 211, wherein said target gene or RNA is from an organism other than *E. coli*.

214. The method of Claim 211, wherein said gene product is from an organism other than *E. coli*.

215. The method of Claim 211, wherein said target is a messenger RNA molecule and said activity is translation of said messenger RNA.

5           216. The method of Claim 211, wherein said compound is a nucleic acid and said activity is translation of said gene product.

217. The method of Claim 211, wherein said target is a gene and said activity is transcription of said gene.

10           218. The method of Claim 211, wherein said target is a nontranslated RNA and said activity is processing or folding of said nontranslated RNA or assembly of said nontranslated RNA into a protein/RNA complex.

219. The method of Claim 211, wherein said target gene is a messenger RNA molecule encoding a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOS.: 42398-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOS: 42398-78581.

220. The method of Claim 11, wherein said target gene comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397; a nucleic acid which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions.

221. A compound or nucleic acid identified using the method of Claim 211.

222. A method for identifying a compound which reduces the activity or level of a gene product required for proliferation of a cell comprising:

30           (a) providing a sublethal level of an antisense nucleic acid complementary to a nucleic acid encoding said gene product in a cell to reduce the activity or amount of said gene product in said cell, thereby producing a sensitized cell, wherein said gene product is selected from the group consisting of a gene product having having at least 70% nucleic acid identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.:  
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- 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213;
- (b) contacting said sensitized cell with a compound; and
- (c) determining the degree to which said compound inhibits the growth of said sensitized cell relative to a cell which does not contain said antisense nucleic acid.
223. The method of Claim 222, wherein said determining step comprises determining whether said compound inhibits the growth of said sensitized cell to a greater extent than said compound inhibits the growth of a nonsensitized cell.
224. The method of Claim 222, wherein said sensitized cell is a Gram positive bacterium.
225. The method of Claim 224, wherein said Gram positive bacterium is selected from the group consisting of *Staphylococcus* species, *Streptococcus* species, *Enterococcus* species, *Mycobacterium* species, *Clostridium* species, and *Bacillus* species.
226. The method of Claim 225, wherein said bacterium is *Staphylococcus aureus*.
227. The method of Claim 224, wherein said *Staphylococcus* species is coagulase negative.
228. The method of Claim 226, wherein said bacterium is selected from the group consisting of *Staphylococcus aureus* RN450 and *Staphylococcus aureus* RN4220.
229. The method of Claim 222, wherein said sensitized cell is an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*,

Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile,  
 5 Clostridium perfringens, Coccidioides immitis, Corynebacterium diphtheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium  
 10 tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella choleraesuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella  
 15 boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

- 20 230. The method of Claim 222, wherein said cell is an organism other than *E. coli*.  
 231. The method of Claim 222, wherein said gene product is from an organism other than *E. coli*.  
 232. The method of Claim 222, wherein said antisense nucleic acid is transcribed from an inducible promoter.  
 25 233. The method of Claim 222, further comprising the step of contacting said cell with a concentration of inducer which induces transcription of said antisense nucleic acid to a sublethal level.  
 234. The method of Claim 222, wherein growth inhibition is measured by monitoring optical density of a culture medium.  
 30 235. The method of Claim 222, wherein said gene product is a polypeptide.  
 236. The method of Claim 235, wherein said polypeptide comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs: 42398-78581 and a polypeptide whose activity may be complemented by a  
 35 polypeptide selected from the group consisting of SEQ ID NOs: 42398-78581.

237. The method of Claim 222, wherein said gene product is an RNA.

238. The method of Claim 222, wherein said nucleic acid encoding said gene product comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleic acid identity as determined using  
5 BLASTN version 2.0 with the default parameters to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions.

10 239. A compound identified using the method of Claim 222.

240. A method for inhibiting cellular proliferation comprising introducing a compound with activity against a gene product or a compound with activity against a gene encoding said gene product into a population of cells expressing said gene product, wherein said gene product is selected from the group consisting of a gene product having at least 70%  
15 nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a  
20 gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213,  
25 a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene  
30 product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213.

241. The method of Claim 240, wherein said compound is an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213, or a proliferation-inhibiting portion thereof.



242. The method of Claim 240, wherein said proliferation inhibiting portion of one of SEQ ID NOs.: 1-6213 is a fragment comprising at least 10, at least 20, at least 25, at least 30, at least 50 or more than 51 consecutive nucleotides of one of SEQ ID NOs.: 1-6213.

243. The method of Claim 240, wherein said population is a population of Gram positive bacteria.

244. The method of Claim 243, wherein said population of Gram positive bacteria is selected from the group consisting of a population of *Staphylococcus* species, *Streptococcus* species, *Enterococcus* species, *Mycobacterium* species, *Clostridium* species, and *Bacillus* species.

245. The method of Claim 243, wherein said population is a population of *Staphylococcus aureus*.

246. The method of Claim 245, wherein said population is a population of a bacterium selected from the group consisting of *Staphylococcus aureus* RN450 and *Staphylococcus aureus* RN4220.

247. The method of Claim 240, wherein said population is a population of a bacterium selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*,

*Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

248. The method of Claim 240, wherein said population is a population of an organism other than *E. coli*.

5           249. The method of Claim 240, wherein said product of said gene is from an organism other than *E. coli*.

250. The method of Claim 240, wherein said gene product is selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOS.: 42398-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOS.: 42398-78581.

251. The method of Claim 240, wherein said gene comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions.

252. A preparation comprising an effective concentration of an antisense nucleic acid in a pharmaceutically acceptable carrier wherein said antisense nucleic acid is selected from the group consisting of a nucleic acid comprising a sequence having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213 or a proliferation-inhibiting portion thereof, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213 under moderate conditions.

253. The preparation of Claim 252, wherein said proliferation-inhibiting portion of one of SEQ ID NOS.: 1-6213 comprises at least 10, at least 20, at least 25, at least 30, at least 50 or more than 50 consecutive nucleotides of one of SEQ ID NOS.: 1-6213.

254. A method for inhibiting the activity or expression of a gene in an operon which encodes a gene product required for proliferation comprising contacting a cell in a cell

population with an antisense nucleic acid comprising at least a proliferation-inhibiting portion of said operon in an antisense orientation, wherein said gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213.

255. The method of Claim 254, wherein said antisense nucleic acid comprises a nucleotide sequence having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a proliferation inhibiting portion thereof, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, and a nucleic acid which comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions.

256. The method of Claim 254, wherein said cell is selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*,

Corynebacterium diphtheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella choleraesuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

257. The method of Claim 254, wherein said cell is not an *E. coli* cell.

258. The method of Claim 254, wherein said gene is from an organism other than *E. coli*.

259. The method of Claim 254, wherein said cell is contacted with said antisense nucleic acid by introducing a plasmid which transcribes said antisense nucleic acid into said cell population.

260. The method of Claim 254, wherein said cell is contacted with said antisense nucleic acid by introducing a phage which transcribes said antisense nucleic acid into said cell population.

261. The method of Claim 254, wherein said cell is contacted with said antisense nucleic acid by transcribing said antisense nucleic acid from the chromosome of cells in said cell population.

262. The method of Claim 254, wherein said cell is contacted with said antisense nucleic acid by introducing a promoter adjacent to a chromosomal copy of said antisense nucleic acid such that said promoter directs the synthesis of said antisense nucleic acid.

263. The method of Claim 254, wherein said cell is contacted with said antisense nucleic acid by introducing a retron which expresses said antisense nucleic acid into said cell population.

264. The method of Claim 254, wherein said cell is contacted with said antisense nucleic acid by introducing a ribozyme into said cell-population, wherein a binding portion of said ribozyme is complementary to said antisense oligonucleotide.

5 265. The method of Claim 254, wherein said cell is contacted with said antisense nucleic acid by introducing a liposome comprising said antisense oligonucleotide into said cell.

266. The method of Claim 254, wherein said cell is contacted with said antisense nucleic acid by electroporation of said antisense nucleic acid into said cell.

10 267. The method of Claim 254, wherein said antisense nucleic acid has at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence comprising at least 10, at least 20, at least 25, at least 30, at least 50 or more than 50 consecutive nucleotides of one of SEQ ID NOs.: 1-6213.

268. The method of Claim 254 wherein said antisense nucleic acid is a synthetic oligonucleotide.

15 269. The method of Claim 254, wherein said gene comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a  
20 nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions.

270. A method for identifying a gene which is required for proliferation of a cell comprising:

25 (a) contacting a cell with an antisense nucleic acid selected from the group consisting of a nucleic acid at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 or a proliferation-inhibiting portion thereof, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent  
30 conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, wherein said cell is a cell other than the organism from which said nucleic acid was obtained;

(b) determining whether said nucleic acid inhibits proliferation of said cell; and

(c) identifying the gene in said cell which encodes the mRNA which is complementary to said antisense nucleic acid or a portion thereof.

271. The method of Claim 270, wherein said cell is selected from the group consisting of *Staphylococcus* species, *Streptococcus* species, *Enterococcus* species,  
5 *Mycobacterium* species, *Clostridium* species, and *Bacillus* species.

272. The method of Claim 270 wherein said cell is selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*,  
15 *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*,  
20 *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

273. The method of Claim 270, wherein said cell is not *E. coli*.

274. The method of Claim 270, further comprising operably linking said antisense nucleic acid to a promoter which is functional in said cell, said promoter being included in a vector, and introducing said vector into said cell.  
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275. A method for identifying a compound having the ability to inhibit proliferation of a cell comprising:

(a) identifying a homolog of a gene or gene product whose activity or level is inhibited by an antisense nucleic acid in a test cell, wherein said test cell is not the microorganism from which the antisense nucleic acid was obtained, wherein said antisense nucleic acid is selected from the group consisting of a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOs. 1-6213, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213 under moderate conditions;

(b) identifying an inhibitory nucleic acid sequence which inhibits the activity of said homolog in said test cell;

(c) contacting said test cell with a sublethal level of said inhibitory nucleic acid, thus sensitizing said cell;

(d) contacting the sensitized cell of step (c) with a compound; and

(e) determining the degree to which said compound inhibits proliferation of said sensitized cell relative to a cell which does not express said inhibitory nucleic acid.

276. The method of Claim 275, wherein said determining step comprises determining whether said compound inhibits proliferation of said sensitized test cell to a greater extent than said compound inhibits proliferation of a nonsensitized test cell.

277. The method of Claim 275, wherein step (a) comprises identifying a homologous nucleic acid to a gene or gene product whose activity or level is inhibited by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOs. 1-6213 or a nucleic acid encoding a homologous polypeptide to a polypeptide whose activity or level is inhibited by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOs. 1-6213 by using an algorithm selected from the group consisting of BLASTN version 2.0 with the default parameters and FASTA version 3.0t78 algorithm with the default parameters to identify said homologous nucleic acid or said nucleic acid encoding a homologous polypeptide in a database.

278. The method of Claim 275 wherein said step (a) comprises identifying a homologous nucleic acid or a nucleic acid encoding a homologous polypeptide by identifying nucleic acids comprising nucleotide sequences which hybridize to said nucleic acid having at

least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOs. 1-6213 or the complement of the nucleotide sequence of said nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213.

5           279. The method of Claim 275 wherein step (a) comprises expressing a nucleic acid having at least 70% nucleic acid identity as determined using BLASTN version 2.0 with the default parameters to a sequence selected from the group consisting of SEQ ID NOs. 1-6213 in said test cell.

10           280. The method of Claim 275, wherein step (a) comprises identifying a homologous nucleic acid or a nucleic acid encoding a homologous polypeptide in an test cell selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*),  
 15 *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*,  
 20 *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*,  
 25 *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*,  
 30 *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.



281. The method of Claim 275, wherein step (a) comprises identifying a homologous nucleic acid or a nucleic acid encoding a homologous polypeptide in a test cell other than *E. coli*.

5 282. The method of Claim 275, wherein said inhibitory nucleic acid is an antisense nucleic acid.

283. The method of Claim 275, wherein said inhibitory nucleic acid comprises an antisense nucleic acid to a portion of said homolog.

284. The method of Claim 275, wherein said inhibitory nucleic acid comprises an antisense nucleic acid to a portion of the operon encoding said homolog.

10 285. The method of Claim 275, wherein the step of contacting the cell with a sublethal level of said inhibitory nucleic acid comprises directly contacting said cell with said inhibitory nucleic acid.

286. The method of Claim 275, wherein the step of contacting the cell with a sublethal level of said inhibitory nucleic acid comprises expressing an antisense nucleic acid to said homolog in said cell.

15 287. The method of Claim 275, wherein said gene product comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS.: 42398-78581.

20 288. The method of Claim 275, wherein said gene comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions.

289. A compound identified using the method of Claim 275.

290. A method of identifying a compound having the ability to inhibit proliferation comprising:

30 (a) sensitizing a test cell by contacting said test cell with a sublethal level of an antisense nucleic acid, wherein said antisense nucleic acid is selected from the group consisting of a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS. 1-6213 or a portion thereof which inhibits the proliferation of the cell from which said nucleic acid was

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obtained, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditionst;

(b) contacting the sensitized test cell of step (a) with a compound; and

(c) determining the degree to which said compound inhibits proliferation of said sensitized test cell relative to a cell which does not contain said antisense nucleic acid.

291. The method of Claim 290, wherein said determining step comprises determining whether said compound inhibits proliferation of said sensitized test cell to a greater extent than said compound inhibits proliferation of a nonsensitized test cell.

292. A compound identified using the method of Claim 290.

293. The method of Claim 290, wherein said test cell is selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diptheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio*

vulnificans, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

294. The method of Claim 290, wherein the test cell is not *E. coli*.

295. A method for identifying a compound having activity against a biological pathway required for proliferation comprising:

(a) sensitizing a cell by providing a sublethal level of an antisense nucleic acid complementary to a nucleic acid encoding a gene product required for proliferation, wherein said gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213;

(b) contacting the sensitized cell with a compound; and

(c) determining the extent to which said compound inhibits the growth of said sensitized cell relative to a cell which does not contain said antisense nucleic acid.

296. The method of Claim 295, wherein said determining step comprises determining whether said compound inhibits the growth of said sensitized cell to a greater extent than said compound inhibits the growth of a nonsensitized cell.

297. The method of Claim 295, wherein said cell is selected from the group consisting of bacterial cells, fungal cells, plant cells, and animal cells.

298. The method of Claim 295, wherein said cell is a Gram positive bacterium.

299. The method of Claim 298, wherein said Gram positive bacterium is selected from the group consisting of *Staphylococcus* species, *Streptococcus* species, *Enterococcus* species, *Mycobacterium* species, *Clostridium* species, and *Bacillus* species.

5 300. The method of Claim 299, wherein said Gram positive bacterium is *Staphylococcus aureus*.

301. The method of Claim 298, wherein said Gram positive bacterium is selected from the group consisting of *Staphylococcus aureus* RN450 and *Staphylococcus aureus* RN4220.

10 302. The method of Claim 295, wherein said cell is selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*,  
15 *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*,  
20 *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*,  
25 *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of  
30 any of the above species.

303. The method of Claim 295, wherein said cell is not an *E. coli* cell.

304. The method of Claim 295, wherein said gene product is from an organism other than *E. coli*.

305. The method of Claim 295, wherein said antisense nucleic acid is transcribed from an inducible promoter.

306. The method of Claim 305, further comprising contacting the cell with an agent which induces expression of said antisense nucleic acid from said inducible promoter, wherein  
5 said antisense nucleic acid is expressed at a sublethal level.

307. The method of Claim 295, wherein inhibition of proliferation is measured by monitoring the optical density of a liquid culture.

308. The method of Claim 295, wherein said gene product comprises a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the  
10 default parameters to a sequence selected from the group consisting of SEQ ID NOS.: 42398-78581.

309. The method of Claim 295, wherein said nucleic acid encoding said gene product comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using  
15 BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ  
20 ID NOS.: 6214-42397 under moderate conditions.

310. A compound identified using the method of Claim 295.

311. A method for identifying a compound having the ability to inhibit cellular proliferation comprising:

(a) contacting a cell with an agent which reduces the activity or level of a gene  
25 product required for proliferation of said cell, wherein said gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213, a  
30 gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213, a gene product having at least 25% amino acid identity as  
35 determined using FASTA version 3.0t78 with the default parameters to a gene product

whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213;

(b) contacting said cell with a compound; and

(c) determining the degree to which said compound reduces proliferation of said contacted cell relative to a cell which was not contacted with said agent.

312. The method of Claim 311, wherein said determining step comprises determining whether said compound reduces proliferation of said contacted cell to a greater extent than said compound reduces proliferation of cells which have not been contacted with said agent.

313. The method of Claim 311, wherein said cell is selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*,

*Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

5           314. The method of Claim 311, wherein said cell is not an *E. coli* cell.

315. The method of Claim 311, wherein said gene product is from an organism other than *E. coli*.

316. The method of Claim 311, wherein said agent which reduces the activity or level of a gene product required for proliferation of said cell comprises an antisense nucleic acid to a gene or operon required for proliferation.

10           317. The method of Claim 311, wherein said agent which reduces the activity or level of a gene product required for proliferation of said cell comprises a compound known to inhibit growth or proliferation of a cell.

318. The method of Claim 311, wherein said cell contains a mutation which reduces the activity or level of said gene product required for proliferation of said cell.

15           319. The method of Claim 311, wherein said mutation is a temperature sensitive mutation.

320. The method of Claim 311, wherein said gene product comprises a gene product comprises a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

20           321. A compound identified using the method of Claim 311.

322. A method for identifying the biological pathway in which a proliferation-required gene product or a gene encoding a proliferation-required gene product lies comprising:

25           (a) providing a sublethal level of an antisense nucleic acid which inhibits the activity or reduces the level of said gene encoding a proliferation-required gene product or said said proliferation-required gene product in a test cell, wherein said proliferation-required gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group

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consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a  
5 gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product  
10 whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213;

(b) contacting said test cell with a compound known to inhibit growth or proliferation of a cell, wherein the biological pathway on which said compound acts is known; and

15 (c) determining the degree to which said compound inhibits proliferation of said test cell relative to a cell which does not contain said antisense nucleic acid.

323. The method of Claim 322, wherein said determining step comprises determining whether said test cell has a substantially greater sensitivity to said compound than a cell which does not express said sublethal level of said antisense nucleic acid.

20 324. The method of Claim 322, wherein said gene product comprises a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

325. The method of Claim 322, wherein said test cell is selected from the group  
25 consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*,  
30 *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*,



Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,  
 5 Salmonella choleraesuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio  
 10 vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

326. The method of Claim 322, wherein said test cell is not an *E. coli* cell.

327. The method of Claim 322, wherein said gene product is from an organism other than *E. coli*.

15 328. A method for determining the biological pathway on which a test compound acts comprising:

(a) providing a sublethal level of an antisense nucleic acid complementary to a proliferation-required nucleic acid in a cell, thereby producing a sensitized cell, wherein said antisense nucleic acid is selected from the group consisting of a nucleic acid having  
 20 at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 or a proliferation-inhibiting portion thereof, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, and a nucleic acid  
 25 comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions and wherein the biological pathway in which said proliferation-required nucleic acid or a protein encoded by said proliferation-required polypeptide lies is known,

(b) contacting said cell with said test compound; and

30 (c) determining the degree to which said compound inhibits proliferation of said sensitized cell relative to a cell which does not contain said antisense nucleic acid.

329. The method of Claim 328, wherein said determining step comprises determining whether said sensitized cell has a substantially greater sensitivity to said test compound than a cell which does not express said sublethal level of said antisense nucleic acid.

35 330. The method of Claim 328, further comprising:

(d) providing a sublethal level of a second antisense nucleic acid complementary to a second proliferation-required nucleic acid in a second cell, wherein said second proliferation-required nucleic acid is in a different biological pathway than said proliferation-required nucleic acid in step (a); and

5 (e) determining whether said second cell does not have a substantially greater sensitivity to said test compound than a cell which does not express said sublethal level of said second antisense nucleic acid, wherein said test compound is specific for the biological pathway against which the antisense nucleic acid of step (a) acts if said sensitized cell has substantially greater sensitivity to said test compound than said  
10 second cell.

331. The method of Claim 328, wherein said sensitized cell is selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*,  
15 *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*,  
25 *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*,  
30 *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

332. The method of Claim 328, wherein said sensitized cell is not an *E. coli* cell.

333. The method of Claim 328, wherein said proliferation-required nucleic acid is from an organism other than *E. coli*.

334. A compound which inhibits proliferation by interacting with a gene encoding a gene product required for proliferation or with a gene product required for proliferation, wherein said gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213.

335. The compound of Claim 334, wherein said gene product comprises a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

336. The compound of Claim 334, wherein said gene comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions.

337. A method for manufacturing an antibiotic comprising the steps of:

screening one or more candidate compounds to identify a compound that reduces the activity or level of a gene product required for proliferation wherein said gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product  
5 whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose  
10 expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded  
15 by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is  
20 inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 ; and manufacturing the compound so identified.

338. The method of Claim 337, wherein said screening step comprises performing any one of the methods of Claims 205, 211, 222, 275, 290, 295, 311.

339. The method of Claim 337, wherein said gene product comprises a polypeptide  
25 having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

340. A method for inhibiting proliferation of a cell in a subject comprising administering an effective amount of a compound that reduces the activity or level of a gene  
30 product required for proliferation of said cell, wherein said gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid  
35 having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with

the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213.

341. The method of Claim 340 wherein said subject is selected from the group consisting of vertebrates, mammals, avians, and human beings.

342. The method of Claim 340, wherein said gene product comprises a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to an amino acid sequence selected from the group consisting of SEQ ID NOs: 42398-78581.

343. The method of Claim 340, wherein said cell is selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*,

Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

344. The method of Claim 340, wherein said cell is not *E. coli*.

345. The method of Claim 340, wherein said gene product is from an organism other than *E. coli*.

346. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a culture comprising a plurality of strains wherein each strain in said culture overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed;

contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture.

347. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a culture comprising a plurality of strains wherein each strain in said culture overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed;

contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture.

348. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

5 obtaining a culture comprising a plurality of strains wherein each strain in said culture overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed;

10 contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

15 identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture.

349. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

20 obtaining a culture comprising a plurality of strains wherein each strain in said culture overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a  
25 nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of  
30 SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide  
35 sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent

conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed;

contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture.

350. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a culture comprising a plurality of strains wherein each strain in said culture overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed;

contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture.



351. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

5 obtaining a culture comprising a plurality of strains wherein each strain in said culture overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed;

10 contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

15 identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture.

352. The method of Claim 346, 347, 348, 349, 350 or 351, wherein said culture includes at least one strain which does not overexpresses a gene product which is essential for proliferation of said organism.

20 353. The method of Claim 346, 347, 348, 349, 350 or 351, wherein said strains which overexpress said gene products comprise a nucleic acid encoding said gene product which is essential for proliferation of said organism operably linked to a regulatable promoter.

25 354. The method of Claim 346, 347, 348, 349, 350 or 351, wherein said strains which overexpress said gene products a nucleic acid encoding said gene product which is essential for proliferation of said organism operably linked to a constitutive promoter.

355. The method of Claim 346, 347, 348, 349, 350 or 351, wherein said identification step comprises determining the nucleotide sequence of a nucleic acid encoding said gene product in said cell which proliferated more rapidly in said culture.

30 356. The method of Claim 346, 347, 348, 349, 350 or 351, wherein said identification step comprises performing an amplification reaction to identify the nucleic acid encoding said gene product in said cell which proliferated more rapidly in said cell culture.

357. The method of Claim 356, wherein the products of said amplification reaction are labeled with a detectable dye.

358. The method of Claim 346, 347, 348, 349, 350 or 351, wherein said identification step comprises performing a hybridization procedure.

359. The method of Claim 346, 347, 348, 349, 350 or 351, wherein said identification step comprises contacting a nucleic acid array with a nucleic acid encoding said gene product in said cell which proliferated more rapidly in said cell culture.

360. The method of Claim 346, 347, 348, 349, 350 or 351, wherein said organism is selected from the group consisting of bacteria, fungi, and protozoa.

361. The method of Claim 346, 347, 348, 349, 350 or 351, wherein said culture is a culture of an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

362. The method of Claim 346, 347, 348, 349, 350 or 351, wherein said compound is obtained from a library of natural compounds.

363. The method of Claim 346, 347, 348, 349, 350 or 351, wherein said compound is obtained from a library of synthetic compounds.

364. The method of Claim 346, 347, 348, 349, 350 or 351, wherein said compound is present in a crude or partially purified state.

365. The method of Claim 346, 347, 348, 349, 350 or 351, further comprising determining whether said gene product in said strain which proliferated more rapidly in said culture has a counterpart in at least one other organism.

366. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining an array of strains on a solid growth medium wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed;

contacting said array of strains with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

identifying the gene product which is overexpressed in a strain which proliferated more rapidly on said solid medium.

367. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining an array of strains on a solid growth medium wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed;

contacting said array of strains with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

identifying the gene product which is overexpressed in a strain which proliferated more rapidly on said solid medium.

368. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

5 obtaining an array of strains on a solid growth medium wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed;

10 contacting said array of strains with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

15 identifying the gene product which is overexpressed in a strain which proliferated more rapidly on said solid medium.

369. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

20 obtaining an array of strains on a solid growth medium wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product  
25 encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product having at least 25% amino acid identity as determined using  
30 FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product  
35 encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide

sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed;

5           contacting said array of strains with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts;

10           and

          identifying the gene product which is overexpressed in a strain which proliferated more rapidly on said solid medium.

370. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

15           obtaining an array of strains on a solid growth medium wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide

20           sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide

25           sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed;

          contacting said array of strains with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which

30           overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts;

          and

          identifying the gene product which is overexpressed in a strain which proliferated more rapidly on said solid medium.

371. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

5 obtaining an array of strains on a solid growth medium wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed;

10 contacting said array of strains with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts;

15 and

identifying the gene product which is overexpressed in a strain which proliferated more rapidly on said solid medium.

372. The method of Claim 366, 367, 368, 369, 370 or 371, wherein at least one strain in said array does not overexpresses a gene product which is essential for proliferation of said organism.

20

373. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

25 obtaining a plurality of cultures, wherein each culture comprises a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed;

30 contacting each of said cultures with a different concentration of said compound; and

identifying the gene product which is overexpressed in a strain whose proliferation is inhibited by said compound.

374. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures, wherein each culture comprises a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed;

contacting each of said cultures with a different concentration of said compound; and

identifying the gene product which is overexpressed in a strain whose proliferation is inhibited by said compound.

375. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures, wherein each culture comprises a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed;

contacting each of said cultures with a different concentration of said compound; and

identifying the gene product which is overexpressed in a strain whose proliferation is inhibited by said compound.

376. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures, wherein each culture comprises a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product

whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 1-6213 is overexpressed;

contacting each of said cultures with a different concentration of said compound; and

identifying the gene product which is overexpressed in a strain whose proliferation is inhibited by said compound.

377. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures, wherein each culture comprises a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed;

contacting each of said cultures with a different concentration of said compound; and

identifying the gene product which is overexpressed in a strain whose proliferation is inhibited by said compound.

378. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:



obtaining a plurality of cultures, wherein each culture comprises a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed;

contacting each of said cultures with a different concentration of said compound; and

identifying the gene product which is overexpressed in a strain whose proliferation is inhibited by said compound.

379. The method of Claim 373, 374, 375, 376, 377 or 378, wherein at least one strain in said plurality of cultures does not overexpress a gene product which is essential for proliferation of said organism.

380. A method of profiling a compound's activity comprising:

performing the method of Claim 346 on a first culture using a first compound;

performing the method of Claim 346 on a second culture using a second compound; and

comparing the strains identified in said first culture to the strains identified in said second culture.

381. A method of profiling a compound's activity comprising:

performing the method of Claim 347 on a first culture using a first compound;

performing the method of Claim 347 on a second culture using a second compound; and

comparing the strains identified in said first culture to the strains identified in said second culture.

382. A method of profiling a compound's activity comprising:

performing the method of Claim 348 on a first culture using a first compound;

performing the method of Claim 348 on a second culture using a second compound; and

comparing the strains identified in said first culture to the strains identified in said second culture.

383. A method of profiling a compound's activity comprising:

performing the method of Claim 349 on a first culture using a first compound;

performing the method of Claim 349 on a second culture using a second compound; and

comparing the strains identified in said first culture to the strains identified in said second culture.

5 384. A method of profiling a compound's activity comprising:  
performing the method of Claim 350 on a first culture using a first compound;  
performing the method of Claim 350 on a second culture using a second compound; and

10 comparing the strains identified in said first culture to the strains identified in said second culture.

385. A method of profiling a compound's activity comprising:  
performing the method of Claim 351 on a first culture using a first compound;  
performing the method of Claim 351 on a second culture using a second compound; and

15 comparing the strains identified in said first culture to the strains identified in said second culture.

386. A method of profiling a first compound's activity comprising:  
growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein  
20 each strain in said array overexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed, and wherein said first compound and said second compound inhibit the proliferation of  
25 said organism; and

comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

387. A method of profiling a first compound's activity comprising:  
growing an array of strains on a first solid medium comprising said first  
30 compound and on a second solid medium comprising a second compound, wherein each strain in said array overexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed, and wherein said first  
35 compound and said second compound inhibit the proliferation of said organism; and

comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

388. A method of profiling a first compound's activity comprising:

5 growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein each strain in said array overexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed, and wherein said first compound and said  
10 second compound inhibit the proliferation of said organism; and

comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

389. A method of profiling a first compound's activity comprising:

15 growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein each strain in said array overexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default  
20 parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense  
25 nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product  
30 encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, under moderate conditions, and a gene product whose activity may be  
35 complemented by the gene product whose activity is inhibited by a nucleic acid

comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and

comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

390. A method of profiling a first compound's activity comprising:

growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein each strain in said array overexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and

comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

391. A method of profiling a first compound's activity comprising:

growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein each strain in said array overexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and

comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

392. The method of any one of Claims 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390 or 391, wherein said first compound is present in a crude or partially purified state.

393. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

5 obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is underexpressed;

10 contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress said gene product on which said compound acts; and

15 identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture.

394. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

20 obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is underexpressed;

25 contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress said gene product on which said compound acts; and

30 identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture.

395. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

35 obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising

an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed;

5           contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress said gene product on which said compound acts; and

          identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture.

10       396. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

          obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is underexpressed;

35           contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene

product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress said gene product on which said compound acts; and

5 identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture.

397. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

10 obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is underexpressed;

20 contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress said gene product on which said compound acts; and

25 identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture.

398. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

30 obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOS.: 42938-78581 and a polypeptide

whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is underexpressed;

5                   contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress said gene product on which said compound acts; and

                  identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture.

10           399. The method of Claim 393, 394, 395, 396, 397 or 398, wherein at least one strain in said culture does not underexpresses a gene product which is essential for proliferation of said organism.

                  400. The method of Claim 393, 394, 395, 396, 397 or 398, wherein said strains which underexpresses said gene products comprise a nucleic acid complementary to at least a portion of a gene encoding said gene product which is essential for proliferation of said organism operably linked to a regulatable promoter.

15           401. The method of Claim 393, 394, 395, 396, 397 or 398, wherein said strains which underexpress said gene products express an antisense nucleic acid complementary to at least a portion of a gene encoding said gene product which is essential for proliferation of said organism, wherein expression of said antisense nucleic acid reduces expression of said gene product in said strain.

                  402. The method of Claim 393, 394, 395, 396, 397 or 398, wherein said identification step comprises determining the nucleotide sequence of a nucleic acid encoding said gene product in said strain which proliferated more slowly.

25           403. The method of Claim 393, 394, 395, 396, 397 or 398, wherein said identification step comprises performing an amplification reaction to identify the nucleic acid encoding said gene product in said cell which proliferated more slowly.

                  404. The method of Claim 393, 394, 395, 396, 397 or 398, wherein the products of said amplification reaction are labeled with a detectable dye..

30           405. The method of Claim 404, wherein said identification step comprises performing a hybridization procedure.

                  406. The method of Claim 393, 394, 395, 396, 397 or 398, wherein said identification step comprises contacting a nucleic acid array with a nucleic acid encoding said gene product in said cell which proliferated more slowly.



407. The method of Claim 393, 394, 395, 396, 397 or 398, wherein said organism is selected from the group consisting of bacteria, fungi, protozoa.

408. The method of Claim 393, 394, 395, 396, 397 or 398, wherein said culture is a culture of an organism selected from the group consisting of *Acinetobacter baumannii*,  
 5 *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*,  
*Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*,  
*Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called  
*Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*,  
*Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*,  
 10 *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium*  
*botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*,  
*Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus*  
*faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*,  
*Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria*  
 15 *monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*,  
*Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma*  
*pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella*  
*haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*,  
*Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*,  
 20 *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*,  
*Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella*  
*sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*,  
*Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema*  
*pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio*  
 25 *vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of  
 any of the above species.

409. The method of Claim 393, 394, 395, 396, 397 or 398, wherein said compound is obtained from a library of natural compounds.

410. The method of Claim 393, 394, 395, 396, 397 or 398, wherein said compound  
 30 is obtained from a library of synthetic compounds.

411. The method of Claim 393, 394, 395, 396, 397 or 398, wherein said compound is present in a crude or partially purified state.

412. The method of Claim 393, 394, 395, 396, 397 or 398, further comprising  
 determining whether said gene product in said strain which proliferated more slowly in said  
 35 culture has a counterpart in at least one other organism.

413. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures, each culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is underexpressed;

contacting each of said cultures with a different concentration of said compound; and

identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

414. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures, each culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is underexpressed;

contacting each of said cultures with a different concentration of said compound; and

identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

415. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures, each culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed;

contacting each of said cultures with a different concentration of said compound; and

identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

416. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

5 obtaining a plurality of cultures, each culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is underexpressed;

contacting each of said cultures with a different concentration of said compound; and

identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

417. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

35 obtaining a plurality of cultures, each culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the

group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is underexpressed;

contacting each of said cultures with a different concentration of said compound; and

identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

418. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures, each culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOS.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOS.: 42938-78581 is underexpressed;

contacting each of said cultures with a different concentration of said compound; and

identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

419. A method of profiling a compound's activity comprising:

performing the method of Claim 393 on a first culture using a first compound;

performing the method of Claim 393 on a second culture using a second

compound; and

comparing the strains identified in said first culture to the strains identified in said second culture.

420. A method of profiling a compound's activity comprising:

performing the method of Claim 394 on a first culture using a first compound;

performing the method of Claim 394 on a second culture using a second compound; and

comparing the strains identified in said first culture to the strains identified in said second culture.

5 421. A method of profiling a compound's activity comprising:  
performing the method of Claim 395 on a first culture using a first compound;  
performing the method of Claim 395 on a second culture using a second compound; and

10 comparing the strains identified in said first culture to the strains identified in said second culture.

422. A method of profiling a compound's activity comprising  
performing the method of Claim 396 on a first culture using a first compound;  
performing the method of Claim 396 on a second culture using a second compound; and

15 comparing the strains identified in said first culture to the strains identified in said second culture.

423. A method of profiling a compound's activity comprising  
performing the method of Claim 397 on a first culture using a first compound;  
performing the method of Claim 397 on a second culture using a second compound; and

20 comparing the strains identified in said first culture to the strains identified in said second culture.

424. A method of profiling a compound's activity comprising  
performing the method of Claim 398 on a first culture using a first compound;  
25 performing the method of Claim 398 on a second culture using a second compound; and

comparing the strains identified in said first culture to the strains identified in said second culture.

425. A method of profiling a first compound's activity comprising:  
30 growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein said array comprises a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a  
35 nucleic acid comprising a nucleotide sequence selected from the group consisting of

SEQ ID NOs.: 1-6213 is underexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and

comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

5 426. A method of profiling a first compound's activity comprising:

growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein said array comprises a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of an organism, wherein said culture  
10 comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is underexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and

comparing the pattern of strains which grow on said first solid medium with the  
15 pattern of strains which grow on said second solid medium.

427. A method of profiling a first compound's activity comprising:

growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein said array comprises a plurality of strains wherein each strain underexpresses a different  
20 gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and

25 comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

428. A method of profiling a first compound's activity comprising:

growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein said  
30 array comprises a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited  
35 by an antisense nucleic acid comprising a nucleotide sequence selected from the group

consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is underexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and

comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

429. A method of profiling a first compound's activity comprising:

growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein said array comprises a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is underexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and

comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

430. A method of profiling a first compound's activity comprising:

growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein said array comprises a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and

comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

431. The method of any one of Claims 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429 or 430, wherein said first compound is present in a crude or partially purified state.

432. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is underexpressed;

contacting each of said plurality of cultures with a varying concentration of a regulatory agent which regulates the level of expression of said gene products which are essential for proliferation of said organism; and

identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

433. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a



nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is underexpressed;

contacting each of said plurality of cultures with a varying concentration of a regulatory agent which regulates the level of expression of said gene products which are essential for proliferation of said organism; and

identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

434. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed;

contacting each of said plurality of cultures with a varying concentration of a regulatory agent which regulates the level of expression of said gene products which are essential for proliferation of said organism; and

identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

435. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from

the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is underexpressed;

contacting each of said plurality of cultures with a varying concentration of a regulatory agent which regulates the level of expression of said gene products which are essential for proliferation of said organism; and

identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

436. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is underexpressed;

contacting each of said plurality of cultures with a varying concentration of a regulatory agent which regulates the level of expression of said gene products which are essential for proliferation of said organism; and

identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

437. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a plurality of cultures comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed;

contacting each of said plurality of cultures with a varying concentration of a regulatory agent which regulates the level of expression of said gene products which are essential for proliferation of said organism; and

identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

438. A culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed.

439. A culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed.

440. A culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed.

441. A culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising

a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed.

442. A culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed.

443. A culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed.

444. The culture of Claim 438, 439, 440, 441, 442 or 443, wherein said strains which overexpress said gene products comprise a nucleic acid encoding said gene product which is essential for proliferation of said organism operably linked to a regulatable promoter.

445. The culture of Claim 438, 439, 440, 441, 442 or 443, wherein said strains which overexpress said gene products comprise a nucleic acid encoding said gene product which is essential for proliferation of said organism operably linked to a constitutive promoter.

446. The culture of Claim 438, 439, 440, 441, 442 or 443, wherein said culture is a culture of an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

447. A culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is underexpressed.

448. A culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a

nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is underexpressed.

449. A culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed.

450. A culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is underexpressed.

451. A culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent

conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is underexpressed.

452. A culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOS.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOS: 42938-78581 is underexpressed.

453. The culture of Claim 447, 448, 449, 450, 451 or 452, wherein said strains which underexpress said gene products comprise a nucleic acid encoding said gene product which is essential for proliferation of said organism operably linked to a regulatable promoter.

454. The culture of Claim 447, 448, 449, 450, 451 or 452, wherein said strains which underexpress said gene products comprise a nucleic acid encoding said gene product which is essential for proliferation of said organism operably linked to a constitutive promoter.

455. The culture of Claim 447, 448, 449, 450, 451 or 452, wherein said culture is a culture of an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella*

*sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

456. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the overexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the overexpressed genes, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed;

contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

457. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the overexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the overexpressed genes, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed;

contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene



product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

5 identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

458. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

10 obtaining a culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the overexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the overexpressed genes, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed;

15 contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

20 identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

459. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

25 obtaining a culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the overexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the overexpressed genes, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at

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least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed;

contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

460. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the overexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the overexpressed genes, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a

nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed;

contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

461. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the overexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the overexpressed genes, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOS.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOS.: 42938-78581 is overexpressed;

contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and

identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

462. The method of Claim 456, 457, 458, 459, 460 or 461, wherein the nucleotide sequence of each of the genes encoding an overexpressed gene product has been altered by replacing the native promoters of said genes with promoters which facilitate overexpression of said gene products.

5 463. The method of Claim 456, 457, 458, 459, 460 or 461, wherein the nucleotide sequence of each of the genes encoding an overexpressed gene product has been altered by inserting a regulatory element into the native promoters of said genes with a promoter which facilitates overexpression of said gene products.

10 464. The method of Claim 463, wherein said regulatory element is selected from the group consisting of a regulatable promoter, an operator which is recognized by a repressor, a nucleotide sequence which is recognized by a transcriptional activator, a transcriptional terminator, a nucleotide sequence which introduces a bend in the DNA and an upstream activating sequence.

15 465. The method of Claim 456, 457, 458, 459, 460 or 461, wherein the step of identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene comprises performing an amplification reaction and detecting a unique amplification product corresponding to said gene.

20 466. The method of Claim 462, wherein the native promoter of each of the genes encoding a gene product essential for proliferation is replaced with the same promoter.

467. The method of Claim 462, wherein the native promoters of the genes encoding gene products essential for proliferation are replaced with a plurality of promoters selected to give a desired expression level for each gene product.

25 468. The method of Claim 462, wherein said promoters which replaced the native promoters in each strain comprise regulatable promoters.

469. The method of Claim 462, wherein said promoters which replaced the native promoters in each strain each strain comprise constitutive promoters.

470. The method of Claim 456, 457, 458, 459, 460 or 461, wherein said organism is selected from the group consisting of bacteria, fungi, and protozoa.

30 471. The method of Claim 456, 457, 458, 459, 460 or 461, wherein said culture is a culture of an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called  
35 *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*,

*Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*,  
*Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium*  
*botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*,  
*Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus*  
5 *faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*,  
*Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria*  
*monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*,  
*Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma*  
*pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella*  
10 *haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*,  
*Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*,  
*Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*,  
*Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella*  
*sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*,  
15 *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema*  
*pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio*  
*vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of  
any of the above species.

472. A method for identifying the gene product on which a compound which inhibits  
20 proliferation of an organism acts comprising:

obtaining a culture comprising a plurality of strains wherein each strain  
underexpresses a different gene product which is essential for proliferation of said  
organism and wherein the nucleotide sequence of each of the underexpressed genes has  
been altered so as to include a nucleotide sequence which can be used to generate a  
25 unique product corresponding to each of the underexpressed genes and wherein said  
culture comprises a strain in which a gene product whose activity or level is inhibited  
by a nucleic acid comprising a nucleotide sequence selected from the group consisting  
of SEQ ID NOs.: 1-6213 is underexpressed;

contacting said culture with a sufficient concentration of said compound to  
30 inhibit the proliferation of strains of said organism which underexpress said gene  
product on which said compound acts, such that strains which underexpress said gene  
product on which said compound acts proliferate more slowly than strains which do not  
underexpress the gene product on which said compound acts; and

identifying the gene product which is underexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

5 473. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the underexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a  
10 unique product corresponding to each of the underexpressed genes and wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is underexpressed;

contacting said culture with a sufficient concentration of said compound to  
15 inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress the gene product on which said compound acts; and

identifying the gene product which is underexpressed in a strain which  
20 proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

474. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a culture comprising a plurality of strains wherein each strain  
25 underexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the underexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the underexpressed genes, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected  
30 from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed;

contacting said culture with a sufficient concentration of said compound to  
inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not  
35 underexpress the gene product on which said compound acts; and

identifying the gene product which is underexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

475. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the underexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the underexpressed genes, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is underexpressed;

contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress the gene product on which said compound acts; and

identifying the gene product which is underexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

5 476. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the underexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a  
10 unique product corresponding to each of the underexpressed genes, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using  
15 BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is underexpressed;  
20

contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not  
25 underexpress the gene product on which said compound acts; and

identifying the gene product which is underexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

30 477. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising:

obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the underexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a  
35 unique product corresponding to each of the underexpressed genes, wherein said



5 culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is underexpressed;

10 contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress the gene product on which said compound acts; and

identifying the gene product which is underexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

15 478. The method of Claim 472, 473, 474, 475, 476 or 477, wherein the nucleotide sequence of each of the genes encoding an underexpressed gene product has been altered by replacing the native promoters of said genes with promoters which facilitate underexpression of said gene products.

20 479. The method of Claim 472, 473, 474, 475, 476 or 477, wherein the nucleotide sequence of each of the genes encoding an underexpressed gene product has been altered by inserting a regulatory element into the native promoters of said genes with a promoter which facilitates underexpression of said gene products.

25 480. The method of Claim 479, wherein said regulatory element is selected from the group consisting of a regulatable promoter, an operator which is recognized by a repressor, a nucleotide sequence which is recognized by a transcriptional activator, a transcriptional terminator, a nucleotide sequence which introduces a bend in the DNA and an upstream activating sequence.

30 481. The method of Claim 472, 473, 474, 475, 476 or 477, wherein the step of identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture by detecting the unique product corresponding to said gene comprises performing an amplification reaction and detecting a unique amplification product corresponding to said gene.

482. The method of Claim 478, wherein the native promoter of each of the genes encoding a gene product essential for proliferation is replaced with the same promoter.

483. The method of Claim 478, wherein the native promoters of the genes encoding gene products essential for proliferation are replaced with a plurality of promoters selected to give a desired expression level for each gene product.

484. The method of Claim 478, wherein said promoters which replaced the native promoters in each strain comprise regulatable promoters.

485. The method of Claim 478, wherein said promoters which replaced the native promoters in each strain each strain comprise constitutive promoters.

486. The method of Claim 472, 473, 474, 475, 476 or 477, wherein said organism is selected from the group consisting of bacteria, fungi, and protozoa.

487. The method of Claim 472, 473, 474, 475, 476 or 477, wherein said culture is a culture of an organism selected from the group consisting of *Acinetobacter baumannii*, *Anaplasma marginale*, *Aspergillus fumigatus*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Burkholderia cepacia*, *Burkholderia fungorum*, *Burkholderia mallei*, *Campylobacter jejuni*, *Candida albicans*, *Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida krusei*, *Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium acetobutylicum*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Coccidioides immitis*, *Corynebacterium diphtheriae*, *Cryptococcus neoformans*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Helicobacter pylori*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Pneumocystis carinii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Treponema pallidum*, *Ureaplasma urealyticum*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificans*, *Yersinia enterocolitica*, *Yersinia pestis* and any species falling within the genera of any of the above species.

488. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed or underexpressed;

performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

determining the lengths of the amplification products obtained in said amplification reaction.

489. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed or underexpressed;

performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

determining the lengths of the amplification products obtained in said amplification reaction.

490. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed or underexpressed;

performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

determining the lengths of the amplification products obtained in said amplification reaction.

491. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a

gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed or underexpressed;

performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

determining the lengths of the amplification products obtained in said amplification reaction.

492. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed or underexpressed;

performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode

said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

determining the lengths of the amplification products obtained in said amplification reaction.

493. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed or underexpressed;

performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

determining the lengths of the amplification products obtained in said amplification reaction.

494. The method of Claim 488, 489, 490, 491, 492 or 493, wherein one member of each primer pair for each of said genes is labeled with a detectable dye.

495. The method of Claim 488, 489, 490, 491, 492 or 493, wherein:

said nucleic acid sample is divided into N aliquots; and

said amplification reaction is performed on each aliquot using primer pairs complementary to nucleotide sequences within or adjacent to 1/N of the genes which encode said gene products, wherein one of the members of each primer pair in each

aliquot is labeled with a dye and wherein the dyes on the primers in each aliquot are distinguishable from one another.

5           496. The method of Claim 494, further comprising pooling the amplification products from each of the aliquots prior to determining the lengths of the amplification products.

          497. The method of Claim 488, 489, 490, 491, 492 or 493, wherein the native promoters of said genes which encode said gene products have been replaced with a regulatable promoter and one of the primers in said primer pairs is complementary to a nucleotide sequence within said regulatable promoter.

10           498. The method of Claim 496, wherein the native promoters for each of said genes were replaced with the same regulatable promoter.

          499. The method of Claim 496, wherein more than one regulatable promoter was used to replace the promoters of said genes such that some of said genes are under the control of a different regulatable promoter.

15           500. A method for identifying the target of a compound which inhibits the proliferation of an organism comprising:

          obtaining a first nucleic acid sample comprising nucleic acids from a first culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism and wherein said culture or collection of strains has been contacted with said compound;

          obtaining a second nucleic acid sample comprising nucleic acids from a second culture or collection of strains wherein said culture or collection of strains comprises the same strains as said first culture or collection of strains wherein said second culture or collection of strains has not been contacted with said compound;

          performing a first amplification reaction on said first nucleic acid sample using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains;

          performing a second amplification reaction on said second nucleic acid sample using the same set of primer pairs used in said first amplification reaction;

and comparing the amount of each amplification product in said first amplification reaction to the amount of that amplification product in said second amplification reaction, wherein an increased level of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products and a decreased level of of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products, wherein said first and second cultures or collection of strains comprise a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed or underexpressed.

501. A method for identifying the target of a compound which inhibits the proliferation of an organism comprising:

obtaining a first nucleic acid sample comprising nucleic acids from a first culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism and wherein said culture or collection of strains has been contacted with said compound;

obtaining a second nucleic acid sample comprising nucleic acids from a second culture or collection of strains wherein said culture or collection of strains comprises the same strains as said first culture or collection of strains wherein said second culture or collection of strains has not been contacted with said compound;

performing a first amplification reaction on said first nucleic acid sample using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains;

performing a second amplification reaction on said second nucleic acid sample using the same set of primer pairs used in said first amplification reaction;

and comparing the amount of each amplification product in said first amplification reaction to the amount of that amplification product in said second



amplification reaction, wherein an increased level of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products and a decreased level of of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products, wherein said first and second cultures or collection of strains comprise a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed or underexpressed.

502. A method for identifying the target of a compound which inhibits the proliferation of an organism comprising:

obtaining a first nucleic acid sample comprising nucleic acids from a first culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism and wherein said culture or collection of strains has been contacted with said compound;

obtaining a second nucleic acid sample comprising nucleic acids from a second culture or collection of strains wherein said culture or collection of strains comprises the same strains as said first culture or collection of strains wherein said second culture or collection of strains has not been contacted with said compound;

performing a first amplification reaction on said first nucleic acid sample using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains;

performing a second amplification reaction on said second nucleic acid sample using the same set of primer pairs used in said first amplification reaction;

and comparing the amount of each amplification product in said first amplification reaction to the amount of that amplification product in said second amplification reaction, wherein an increased level of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that

the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products and a decreased level of of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products, wherein said first and second cultures or collection of strains comprise a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed or underexpressed.

503. A method for identifying the target of a compound which inhibits the proliferation of an organism comprising:

obtaining a first nucleic acid sample comprising nucleic acids from a first culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism and wherein said culture or collection of strains has been contacted with said compound;

obtaining a second nucleic acid sample comprising nucleic acids from a second culture or collection of strains wherein said culture or collection of strains comprises the same strains as said first culture or collection of strains wherein said second culture or collection of strains has not been contacted with said compound;

performing a first amplification reaction on said first nucleic acid sample using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains;

performing a second amplification reaction on said second nucleic acid sample using the same set of primer pairs used in said first amplification reaction;

and comparing the amount of each amplification product in said first amplification reaction to the amount of that amplification product in said second amplification reaction, wherein an increased level of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products and a decreased

level of of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products, wherein said first and second cultures or collection of strains comprise a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed or underexpressed.

504. A method for identifying the target of a compound which inhibits the proliferation of an organism comprising:

obtaining a first nucleic acid sample comprising nucleic acids from a first culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism and wherein said culture or collection of strains has been contacted with said compound;

obtaining a second nucleic acid sample comprising nucleic acids from a second culture or collection of strains wherein said culture or collection of strains comprises the same strains as said first culture or collection of strains wherein said second culture or collection of strains has not been contacted with said compound;

performing a first amplification reaction on said first nucleic acid sample using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains;

performing a second amplification reaction on said second nucleic acid sample using the same set of primer pairs used in said first amplification reaction;

and comparing the amount of each amplification product in said first amplification reaction to the amount of that amplification product in said second amplification reaction, wherein an increased level of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products and a decreased level of of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products, wherein said first and second cultures or collection of strains comprise a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed or underexpressed.

505. A method for identifying the target of a compound which inhibits the proliferation of an organism comprising:

obtaining a first nucleic acid sample comprising nucleic acids from a first culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene

product which is required for proliferation of said organism and wherein said culture or collection of strains has been contacted with said compound;

5 obtaining a second nucleic acid sample comprising nucleic acids from a second culture or collection of strains wherein said culture or collection of strains comprises the same strains as said first culture or collection of strains wherein said second culture or collection of strains has not been contacted with said compound;

10 performing a first amplification reaction on said first nucleic acid sample using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains;

15 performing a second amplification reaction on said second nucleic acid sample using the same set of primer pairs used in said first amplification reaction;

20 and comparing the amount of each amplification product in said first amplification reaction to the amount of that amplification product in said second amplification reaction, wherein an increased level of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products and a decreased level of of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products, wherein said first and second culture or collection of strains comprise a strain in which a gene product comprising a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed or underexpressed.

506. The method of Claim 500, 501, 502, 503, 504 or 505, wherein one member of each primer pair for each of said genes is labeled with a detectable dye.

35 507. The method of Claim 500, 501, 502, 503, 504 or 505, wherein the native promoters of said genes which encode said gene products have been replaced with a regulatable

promoter and one of the primers in said primer pairs is complementary to a nucleotide sequence within said regulatable promoter.

508. The method of Claim 500, 501, 502, 503, 504 or 505, wherein the native promoters for each of said genes were replaced with the same regulatable promoter.

5 509. The method of Claim 500, 501, 502, 503, 504 or 505, wherein more than one regulatable promoter was used to replace the promoters of said genes such that some of said genes are under the control of a different regulatable promoter.

510. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

10 obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which transcribe an antisense nucleic acid complementary to a different gene product which is required for proliferation of said organism;

performing an amplification reaction using a set of primer pairs which are  
15 complementary to nucleotide sequences within or adjacent to the nucleic acids which encode said antisense nucleic acids, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said  
20 primer pair is present in said culture or collection of strains; and

determining the lengths of the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed or  
25 underexpressed.

511. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of  
30 strains which transcribe an antisense nucleic acid complementary to a different gene product which is required for proliferation of said organism;

performing an amplification reaction using a set of primer pairs which are  
complementary to nucleotide sequences within or adjacent to the nucleic acids which encode said antisense nucleic acids, wherein the members of said set of primer pairs are  
35 designed such that each primer pair would yield an amplification product having a

length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

5 determining the lengths of the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed or underexpressed.

512. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

10 obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which transcribe an antisense nucleic acid complementary to a different gene product which is required for proliferation of said organism;

15 performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the nucleic acids which encode said antisense nucleic acids, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

20 determining the lengths of the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed or underexpressed.

25 513. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

30 obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which transcribe an antisense nucleic acid complementary to a different gene product which is required for proliferation of said organism;

35 performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the nucleic acids which encode said antisense nucleic acids, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other

primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

determining the lengths of the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed or underexpressed.

514. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which transcribe an antisense nucleic acid complementary to a different gene product which is required for proliferation of said organism;

performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the nucleic acids which encode said antisense nucleic acids, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other



primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

determining the lengths of the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed or underexpressed.

515. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which transcribe an antisense nucleic acid complementary to a different gene product which is required for proliferation of said organism;

performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the nucleic acids which encode said antisense nucleic acids, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

determining the lengths of the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product comprising a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOS.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOS.: 42938-78581 is overexpressed or underexpressed.

516. The method of Claim 510, 511, 512, 513, 514 or 515, wherein one member of each primer pair for each of said genes is labeled with a detectable dye.

517. The method of Claim 510, 511, 512, 513, 514 or 515, wherein:

said nucleic acid sample is divided into N aliquots; and

said amplification reaction is performed on each aliquot using primer pairs complementary to nucleotide sequences within or adjacent to 1/N of the genes which encode said gene products, wherein one of the members of each primer pair in each aliquot is labeled with a dye and wherein the dyes on the primers in each aliquot are distinguishable from one another.

518. The method of Claim 517, further comprising pooling the amplification products from each of the aliquots prior to determining the lengths of the amplification products.

519. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which overexpress or underexpress a different gene product which is required for proliferation of said organism;

performing an amplification reaction using primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein said primer pairs are designed such that each primer pair would yield an amplification product which is distinguishable from the amplification products produced by the other primer pairs on the basis selected from the group consisting of length, detectable label and both length and detectable label if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

identifying the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed or underexpressed.

520. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which overexpress or underexpress a different gene product which is required for proliferation of said organism;

performing an amplification reaction using primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein said primer pairs are designed such that each primer pair would yield an amplification product which is distinguishable from the amplification products produced by the other primer pairs on the a basis selected from the group consisting of length, detectable label and both length and detectable label if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

identifying the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed or underexpressed.

521. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which overexpress or underexpress a different gene product which is required for proliferation of said organism;

performing an amplification reaction using primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein said primer pairs are designed such that each primer pair would yield an amplification product which is distinguishable from the amplification products produced by the other primer pairs on the a basis selected from the group consisting of length, detectable label and both length and detectable label if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

identifying the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed or underexpressed.

522. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of

strains which overexpress or underexpress a different gene product which is required for proliferation of said organism;

5 performing an amplification reaction using primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein said primer pairs are designed such that each primer pair would yield an amplification product which is distinguishable from the amplification products produced by the other primer pairs on the a basis selected from the group consisting of length, detectable label and both length and detectable label if a strain comprising the nucleotide sequences complementary to said primer pair is present in  
10 said culture or collection of strains; and

identifying the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product  
15 whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid  
20 comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid  
25 which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene  
30 product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed or underexpressed.

523. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which overexpress or underexpress a different gene product which is required for proliferation of said organism;

5 performing an amplification reaction using primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein said primer pairs are designed such that each primer pair would yield an amplification product which is distinguishable from the amplification products produced by the other primer pairs on the a basis selected from the group consisting of length, detectable label and both length and detectable label if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

10 identifying the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed or underexpressed.

15 524. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising:

20 obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which overexpress or underexpress a different gene product which is required for proliferation of said organism;

25 performing an amplification reaction using primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein said primer pairs are designed such that each primer pair would yield an amplification product which is distinguishable from the amplification products produced by the other primer pairs on the a basis selected from the group consisting of length, detectable label and both length and detectable label if a strain

30

35

comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and

identifying the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product comprising a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed or underexpressed.

525. The method of Claim 519, 520, 521, 522, 523 or 524, wherein said primer pairs are divided into at least two sets, each primer pair comprises a primer which is labeled with a distinguishable dye, and the distinguishable dye used to label each set of primer pairs is distinguishable from the dye used to label the other sets of primer pairs.

526. The method of Claim 519, 520, 521, 522, 523 or 524, wherein:

said nucleic acid sample is divided into N aliquots; and

said amplification reaction is performed on each aliquot using primer pairs complementary to nucleotide sequences within or adjacent to 1/N of the genes which encode said gene products, wherein one of the members of each primer pair in each aliquot is labeled with a dye and wherein the dyes on the primers in each aliquot are distinguishable from one another.

527. The method of Claim 526, further comprising pooling the amplification products from each of the aliquots prior to determining the lengths of the amplification products.

528. The method of Claim 519, 520, 521, 522, 523 or 524, wherein the native promoters of said genes which encode said gene products have been replaced with a regulatable promoter and one of the primers in said primer pairs is complementary to a nucleotide sequence within said regulatable promoter.

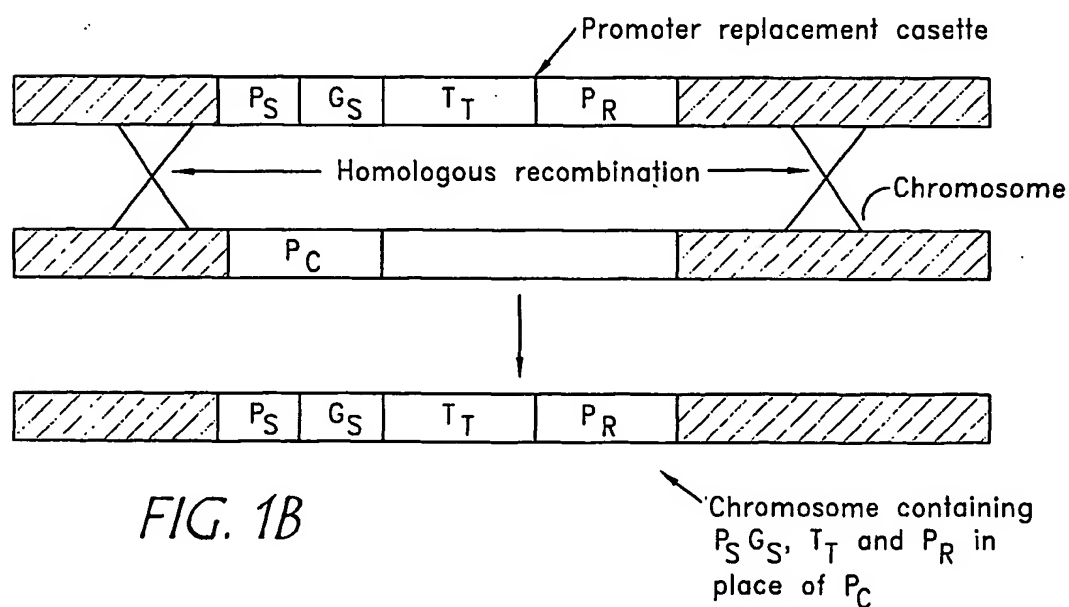
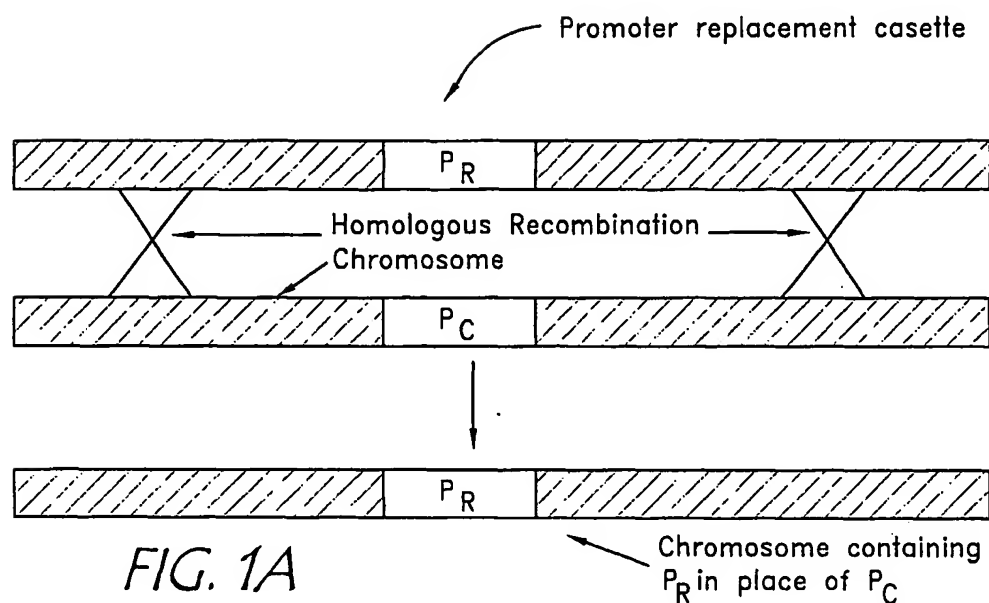
529. The method of Claim 528, wherein the native promoters for each of said genes were replaced with the same regulatable promoter.

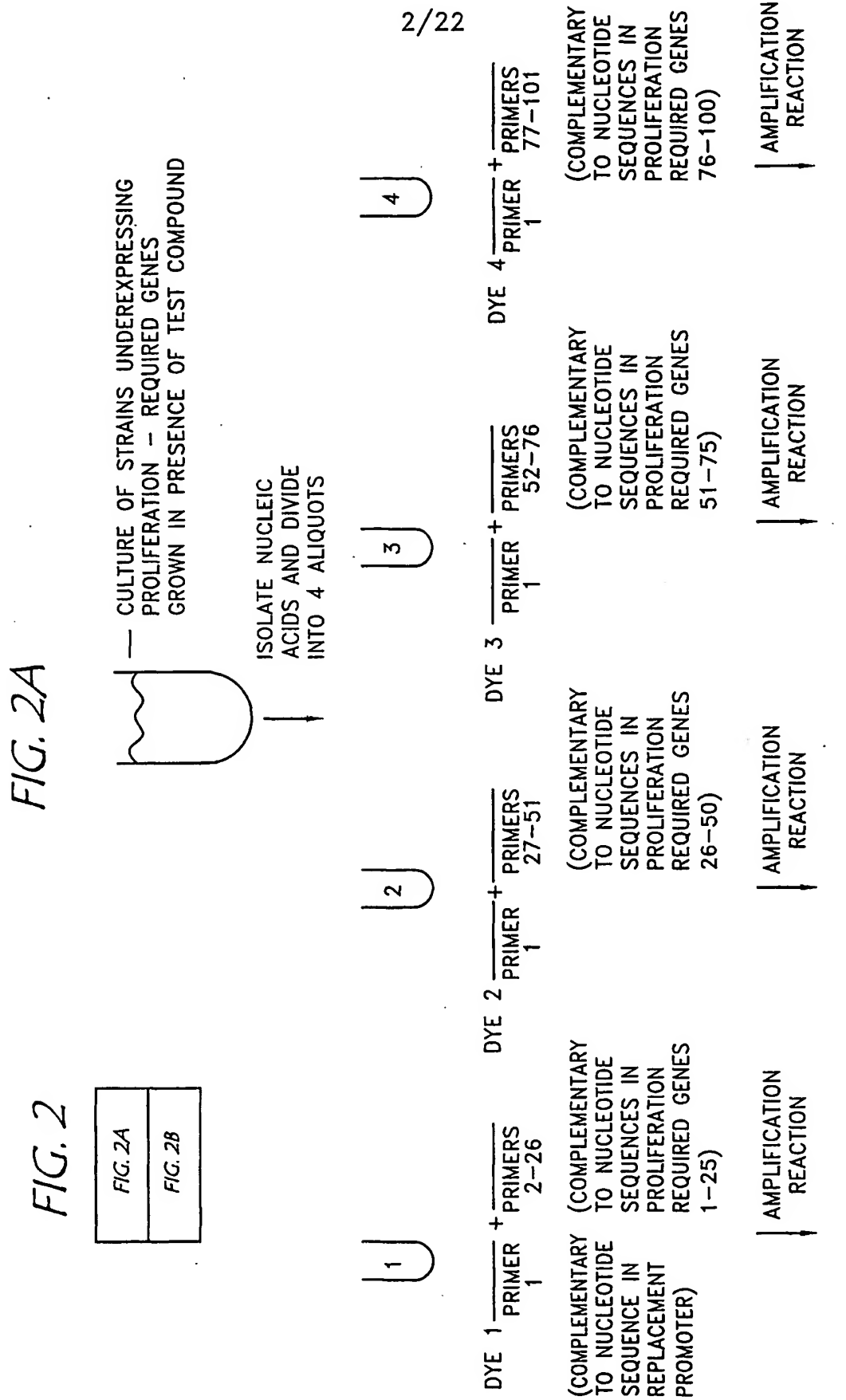
530. The method of Claim 528, wherein more than one regulatable promoter was used to replace the promoters of said genes such that some of said genes are under the control of a different regulatable promoter.

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 $P_R$  = Regulatable promoter $P_C$  = Chromosomal promoter

▨ = Homology region

 $P_S$  = Promoter operably linked to gene $G_S$  = Gene encoding selectable or identifiable marker $T_T$  = Transcriptional terminator





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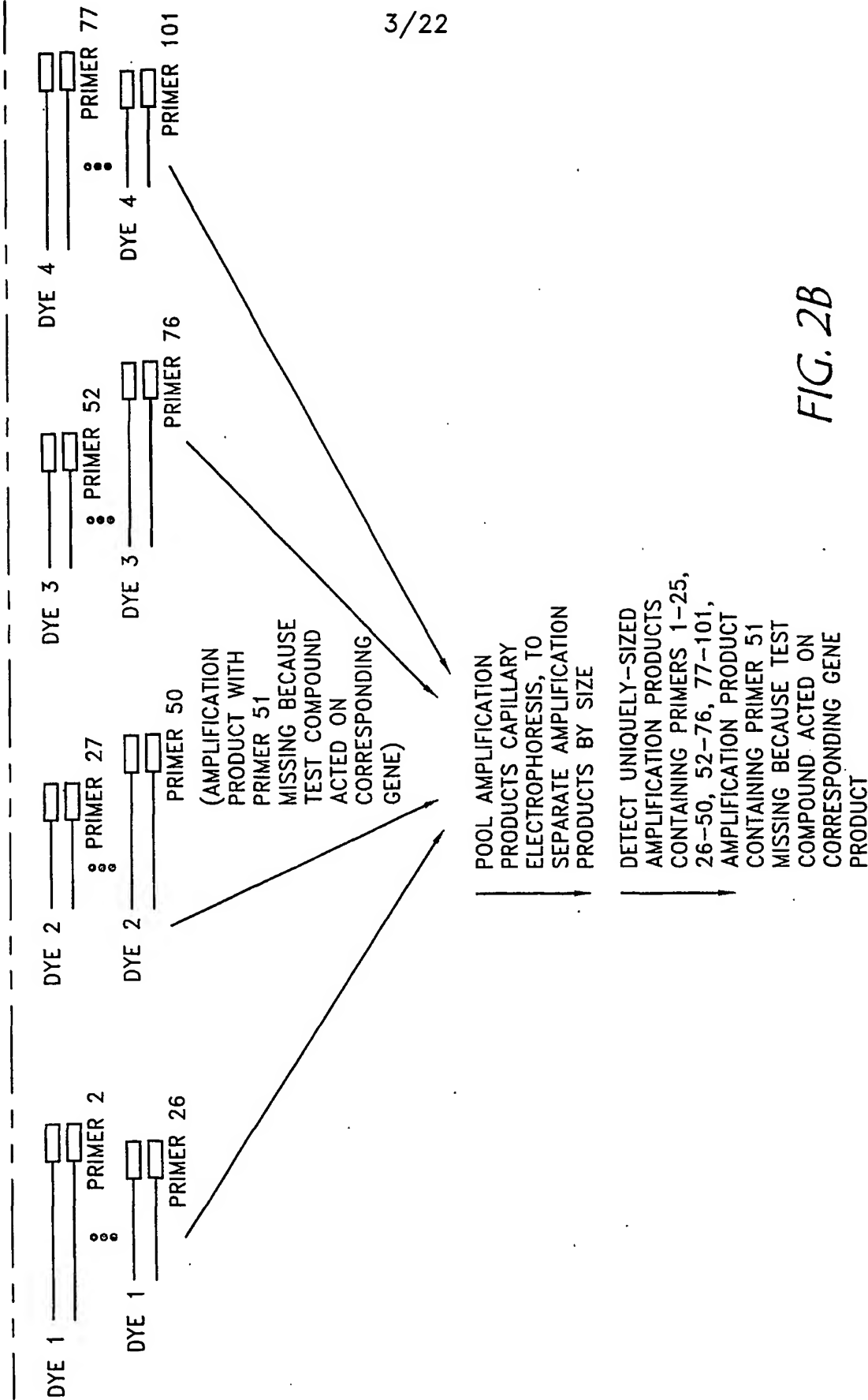


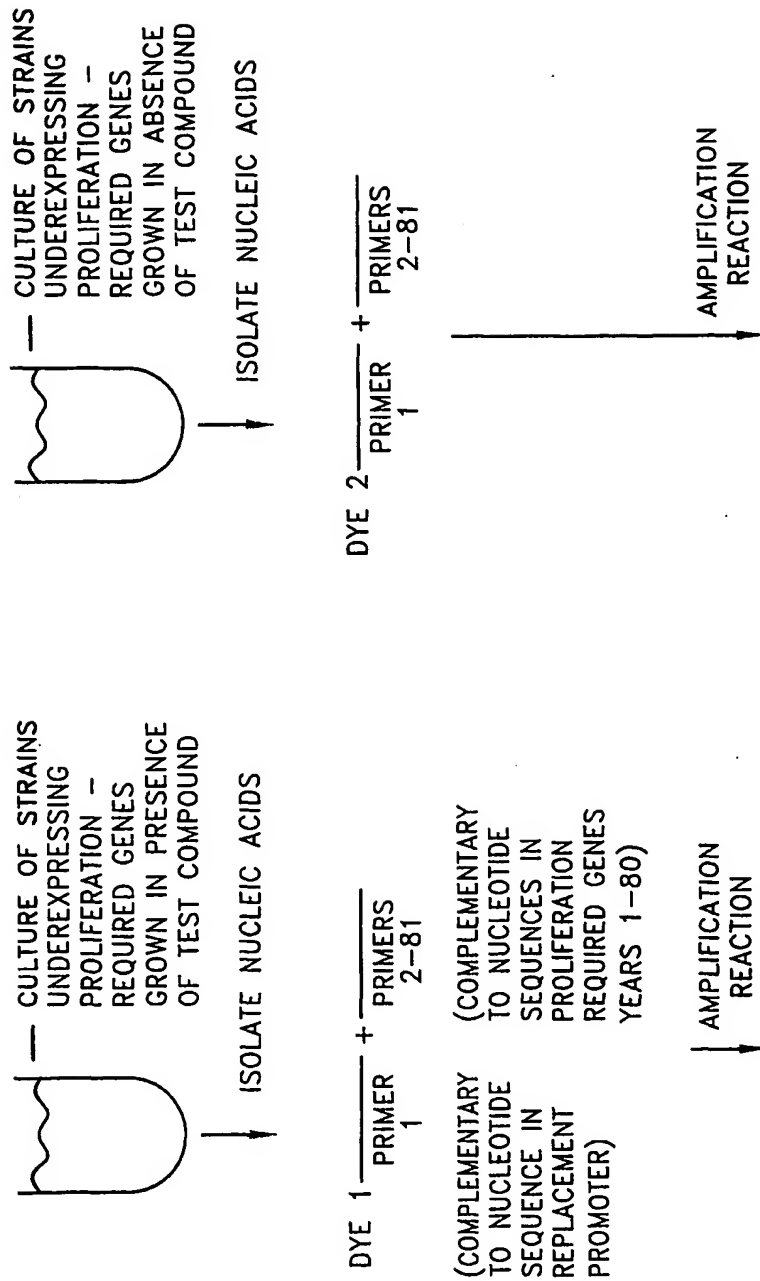
FIG. 2B

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FIG. 3

FIG. 3A
FIG. 3B

FIG. 3A



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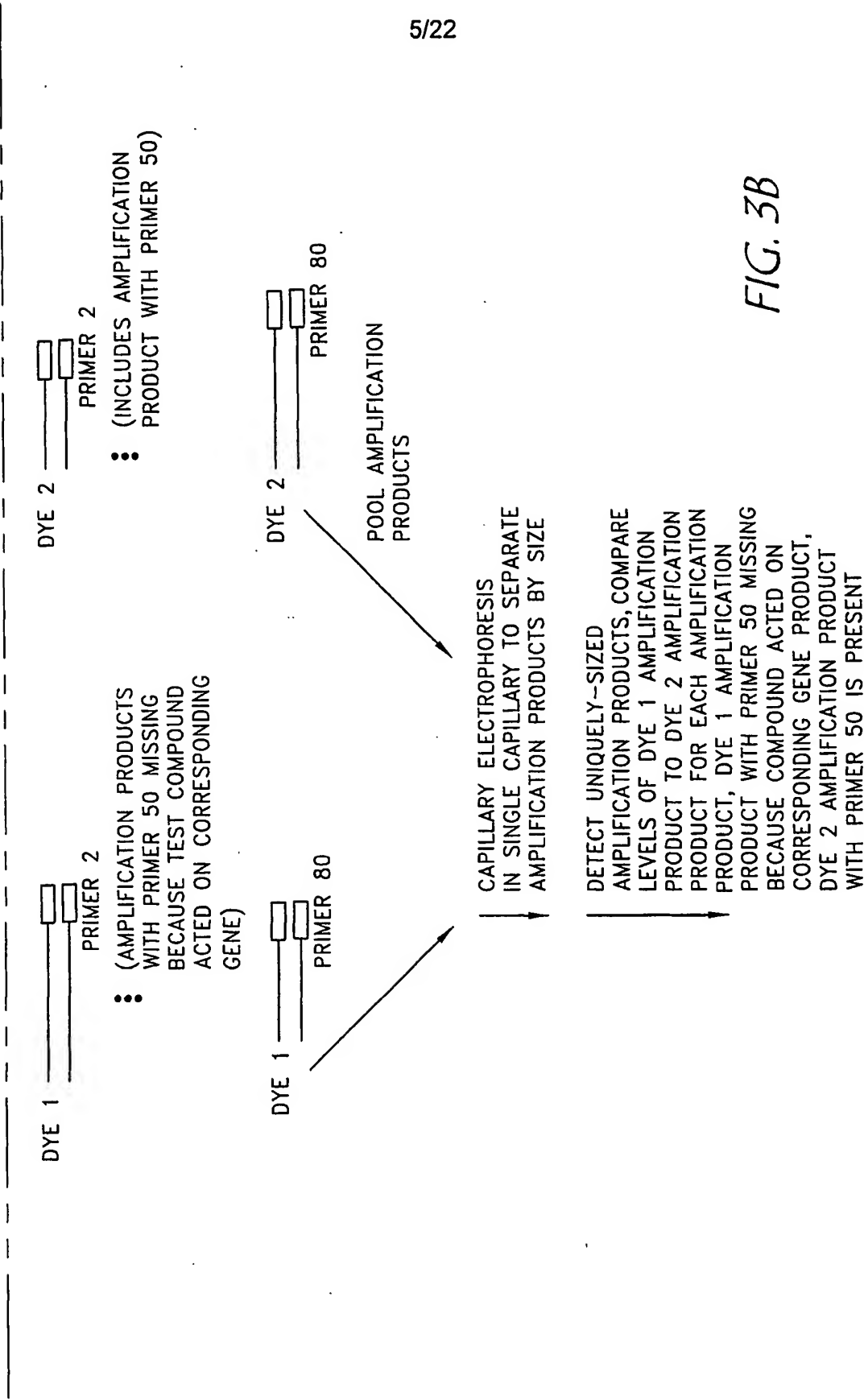
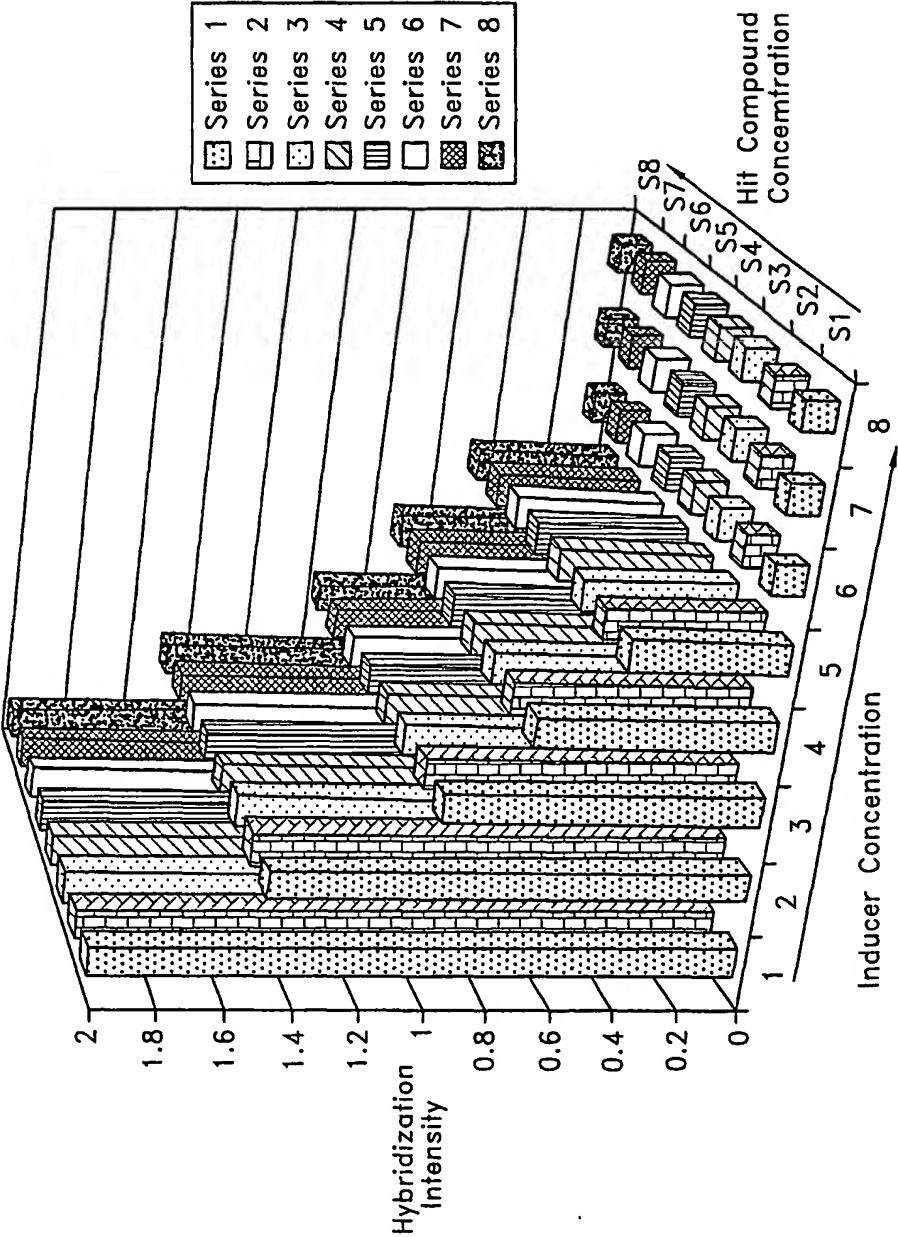


FIG. 3B

FIG. 4

Hypothetical 3D Matrix Hybridization Results for Nonspecific Clones



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Hypothetical 3 D Matrix Hybridization Results for A Specific Clone

FIG. 5

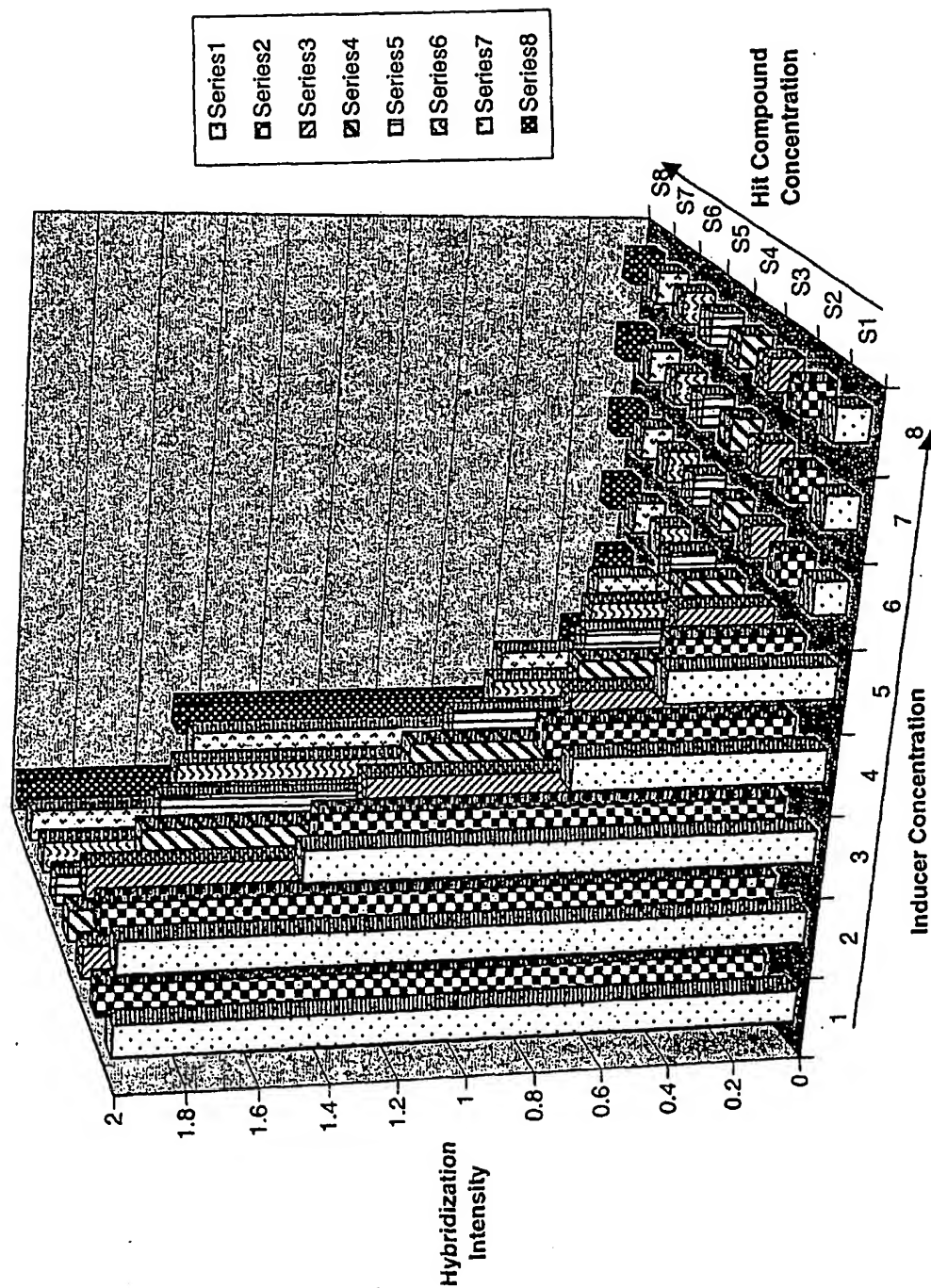
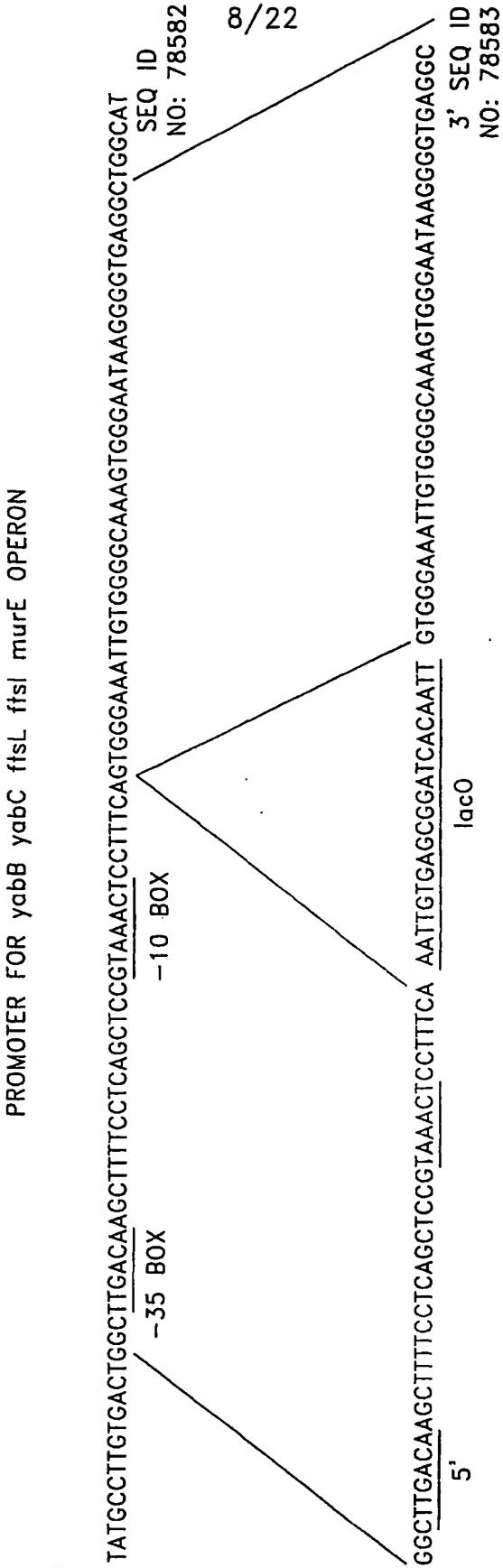


FIG. 6



OLIGO USED FOR lacO INSERTION

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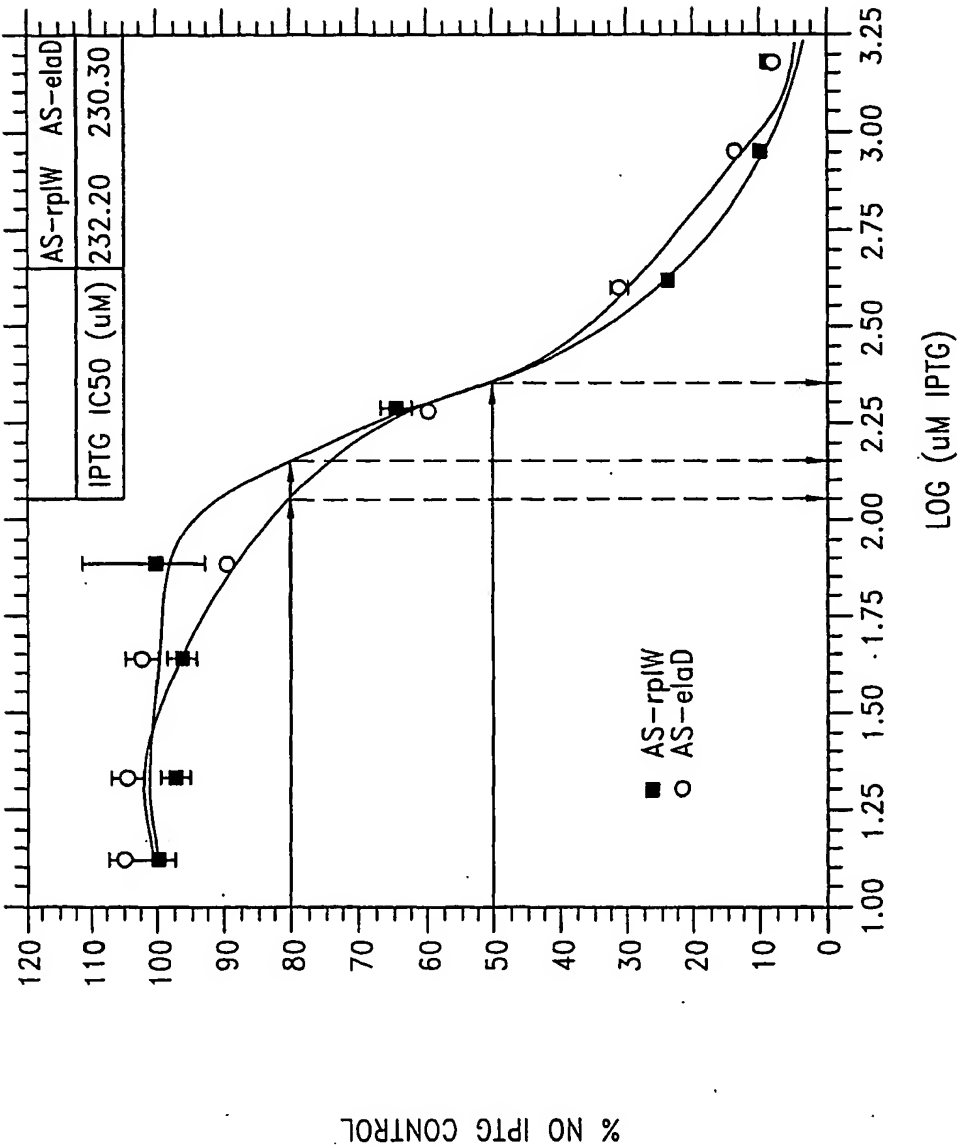


FIG. 7

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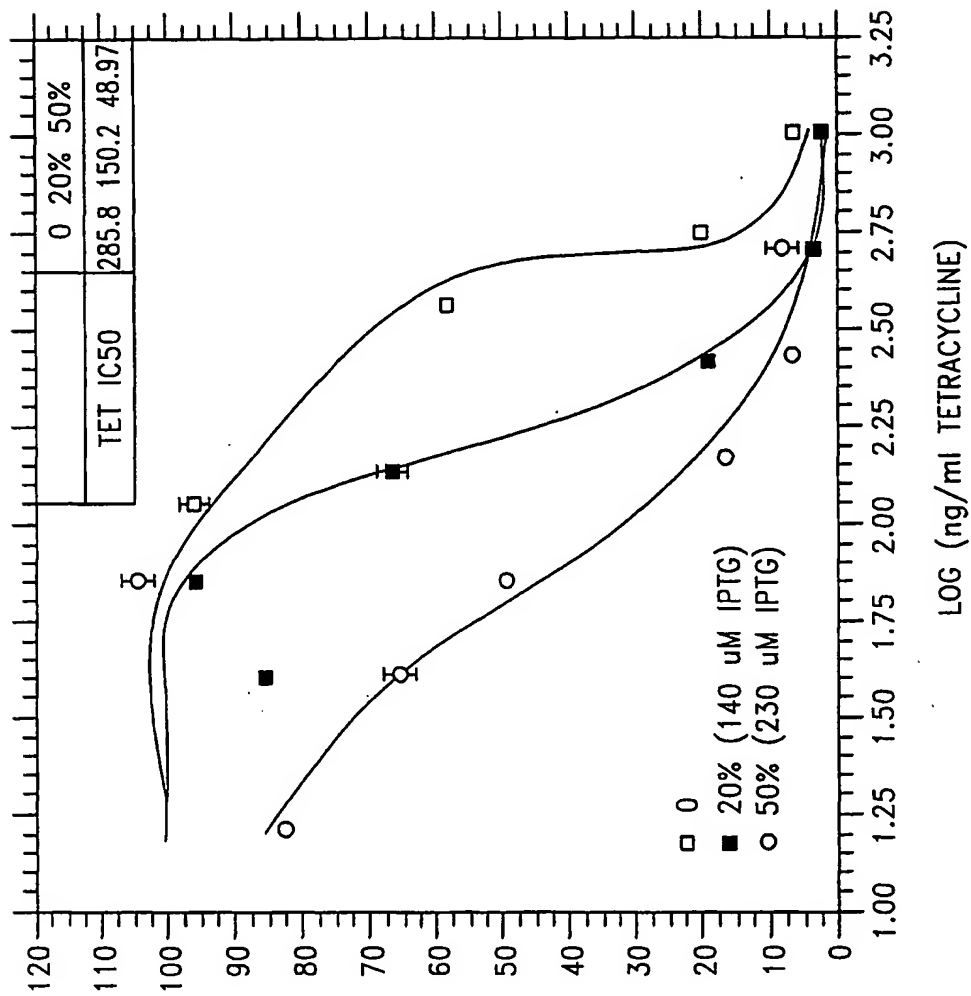


FIG. 8A



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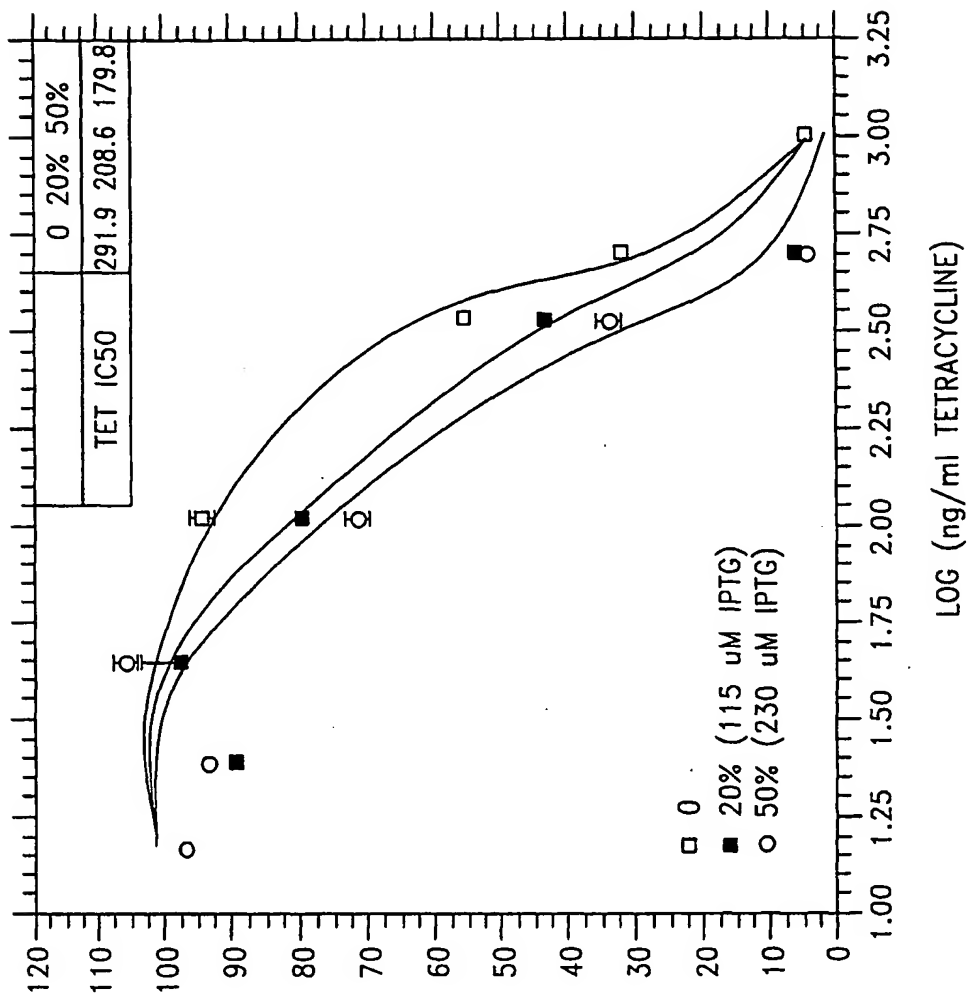


FIG. 8B

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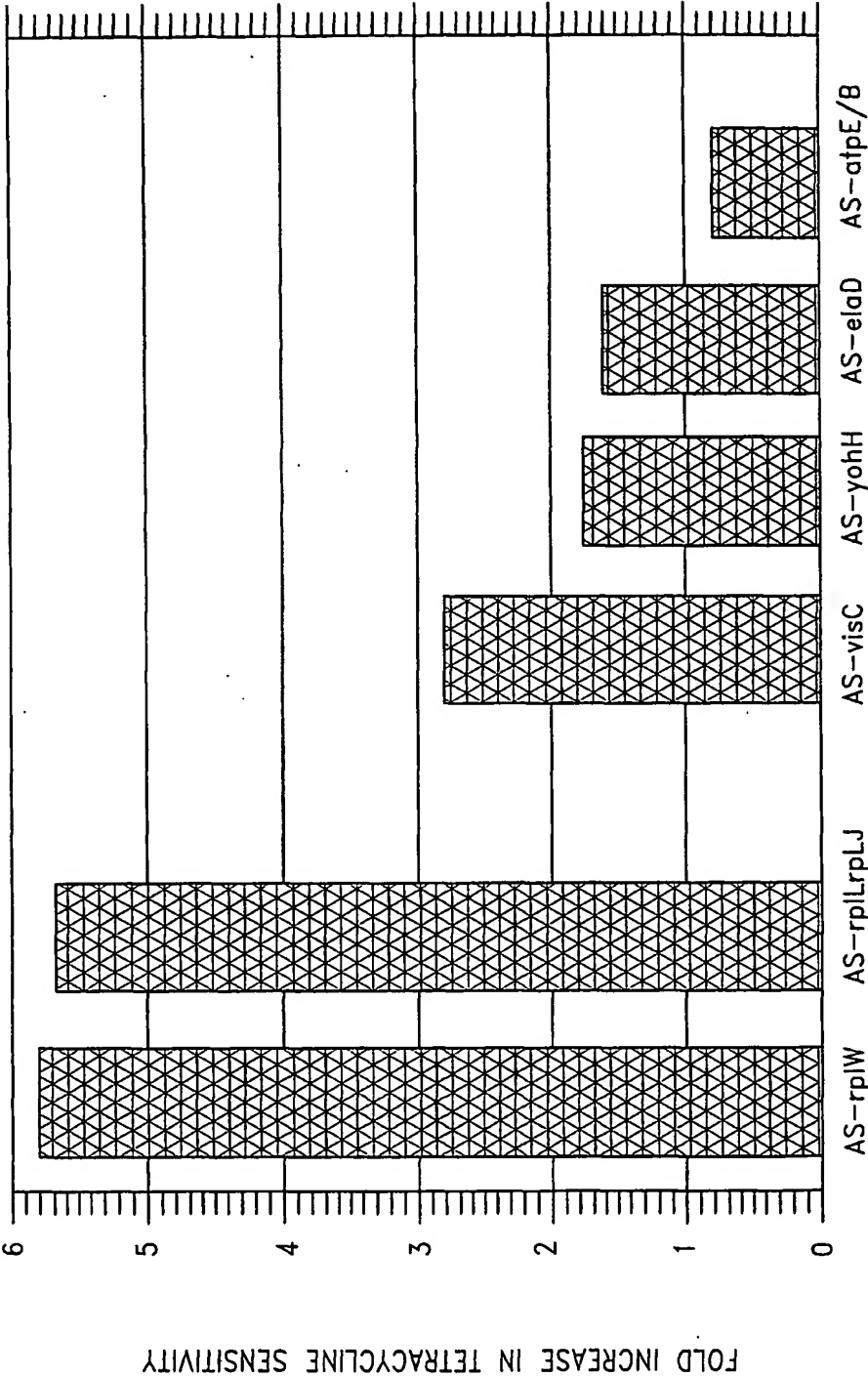
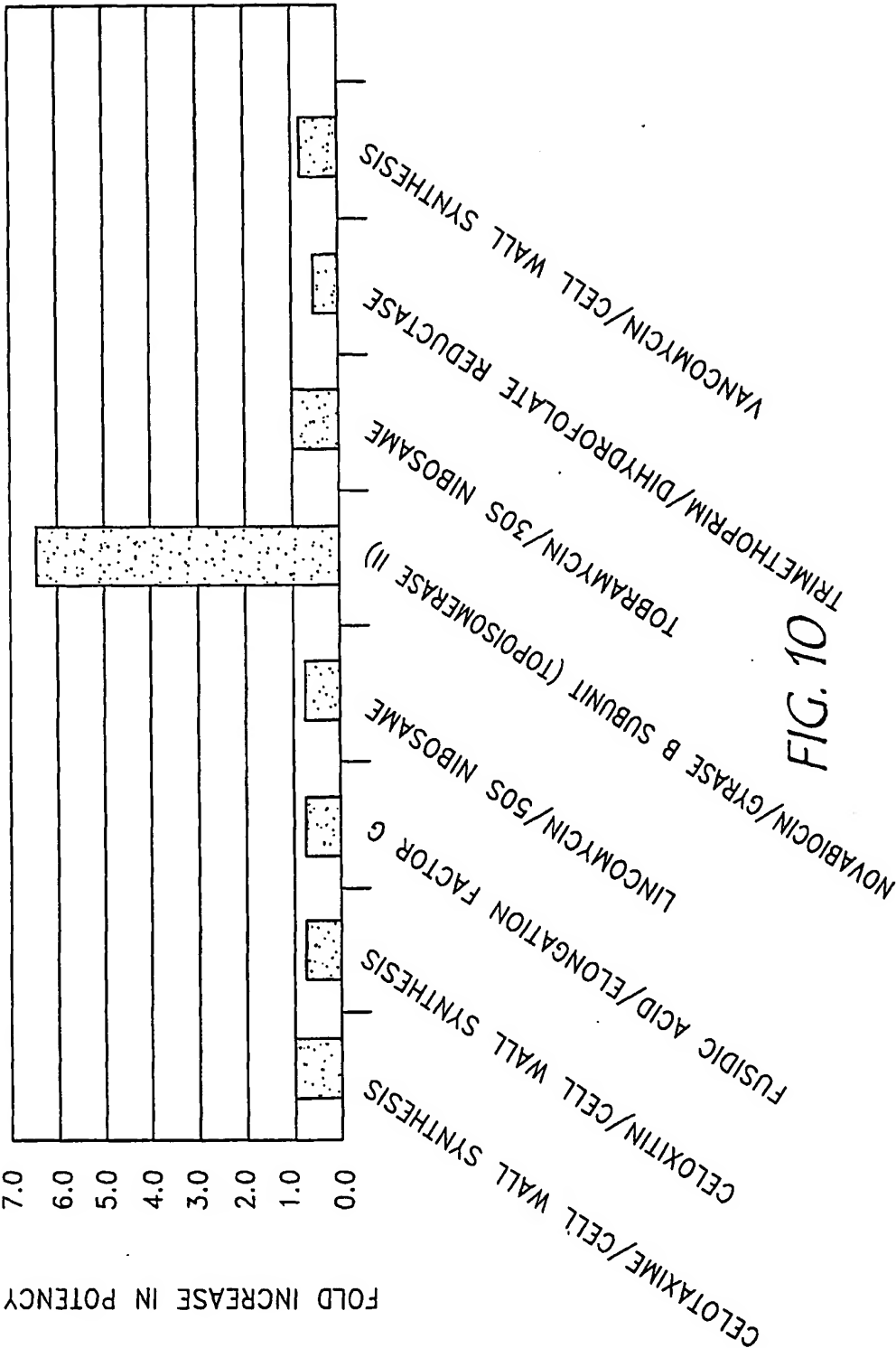


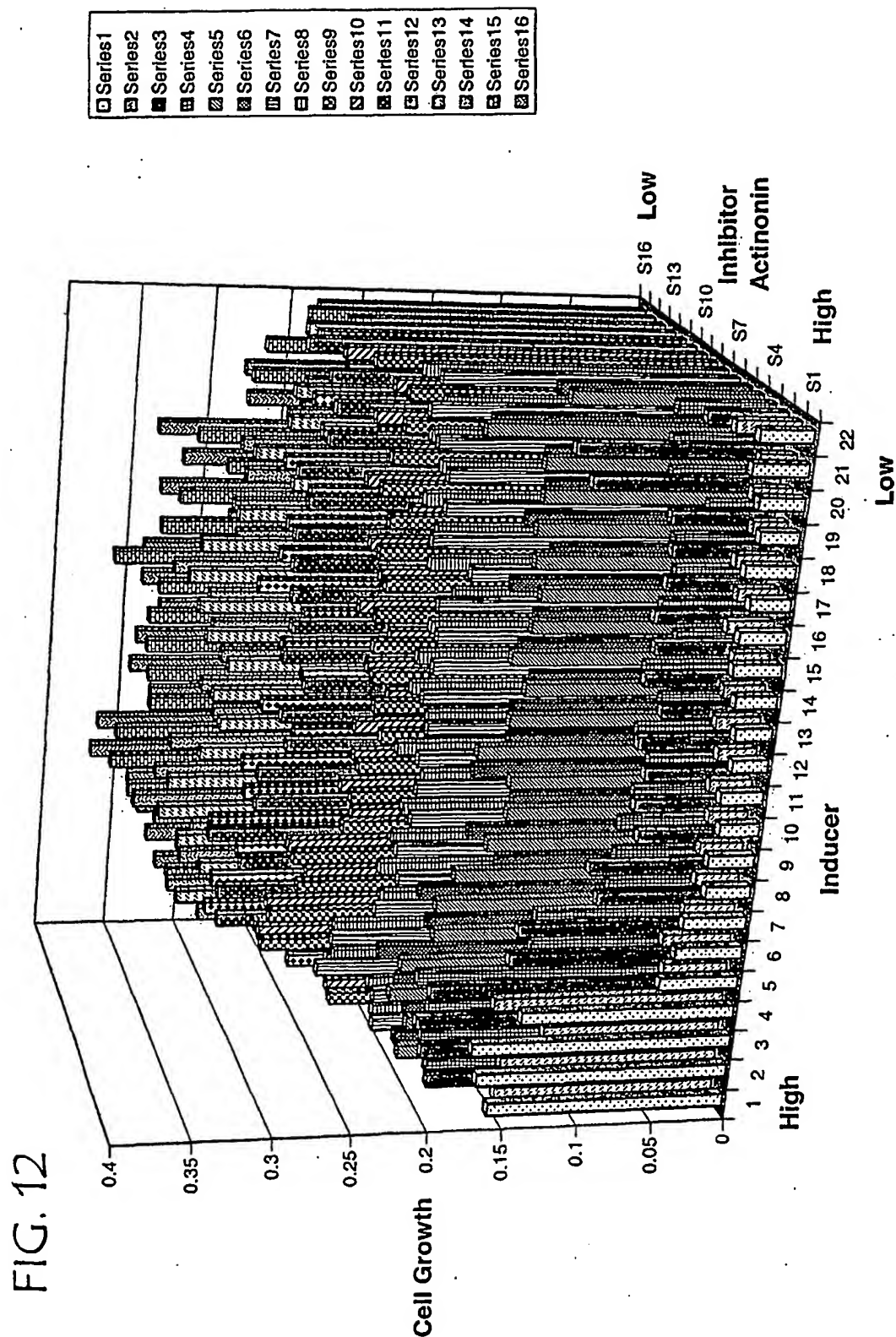
FIG. 9

THE SELECTIVE SENSITIZATION TO AN ANTIBIOTIC INHIBITING GYRASE B SUBUNIT ACTIVITY FOLLOWING THE INDUCTION OF AN ANTISENSE CONSTRUCT TO THE B SUBUNIT OF GYRASE.



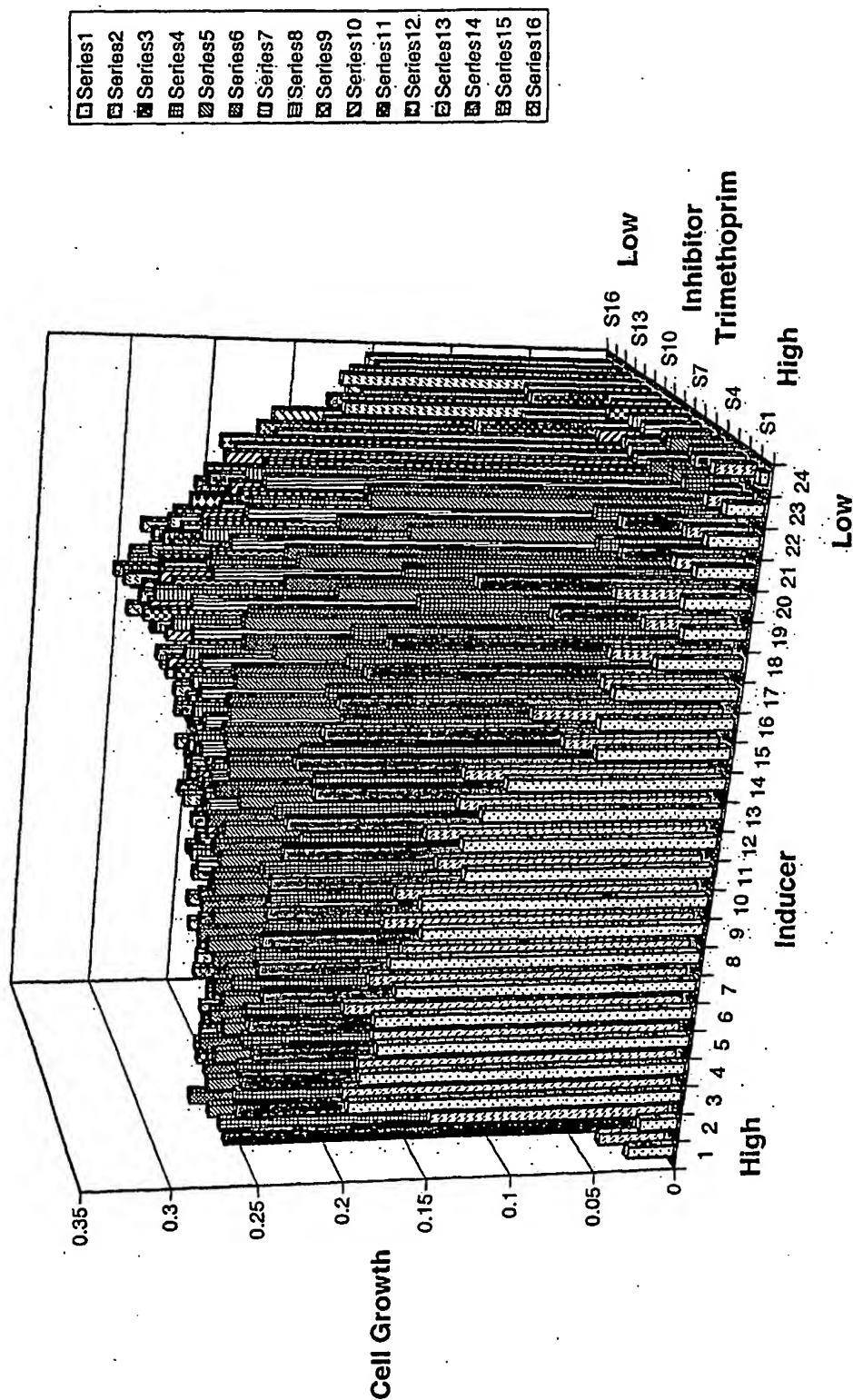


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FIG. 13



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FIG. 14

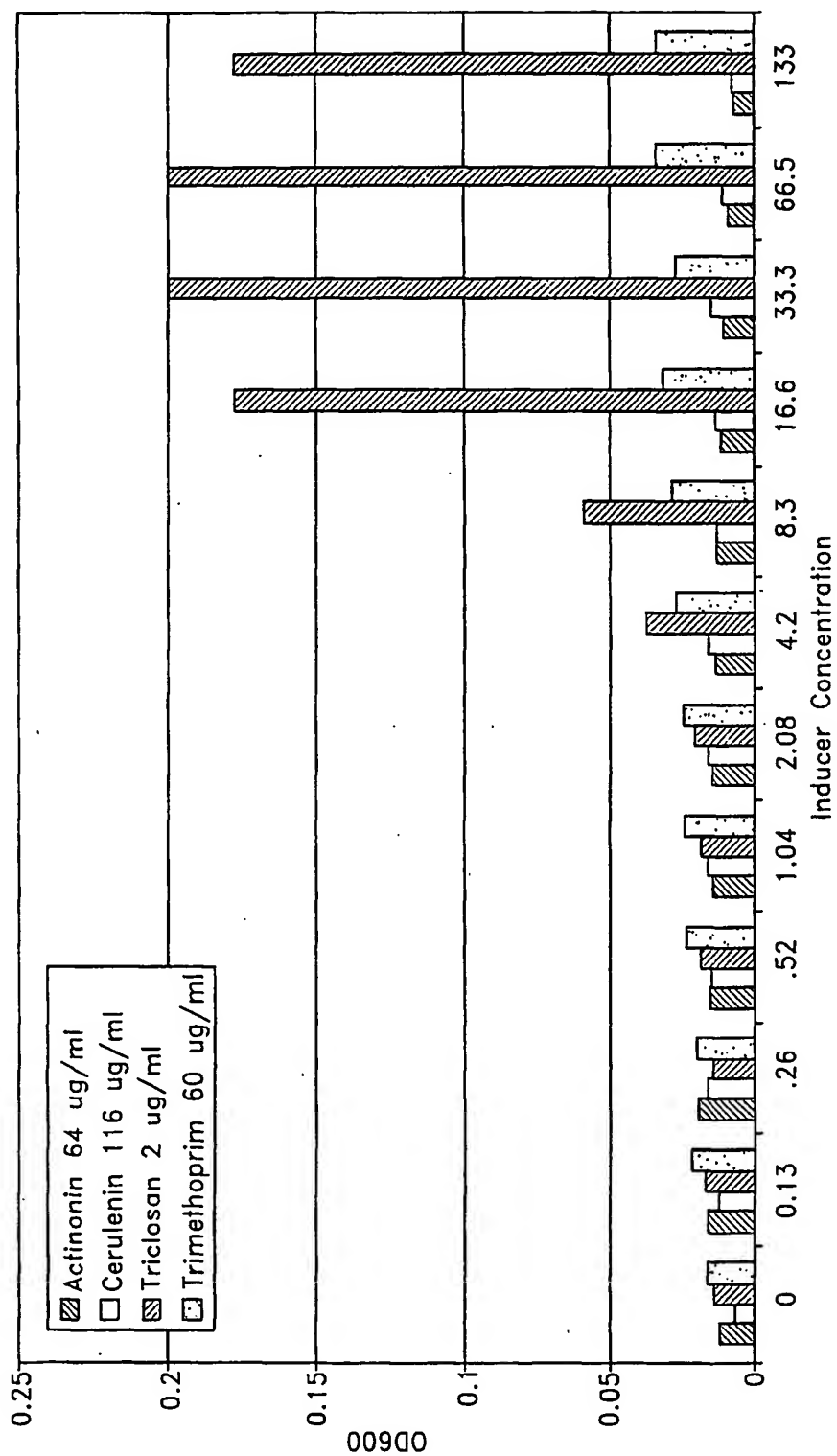
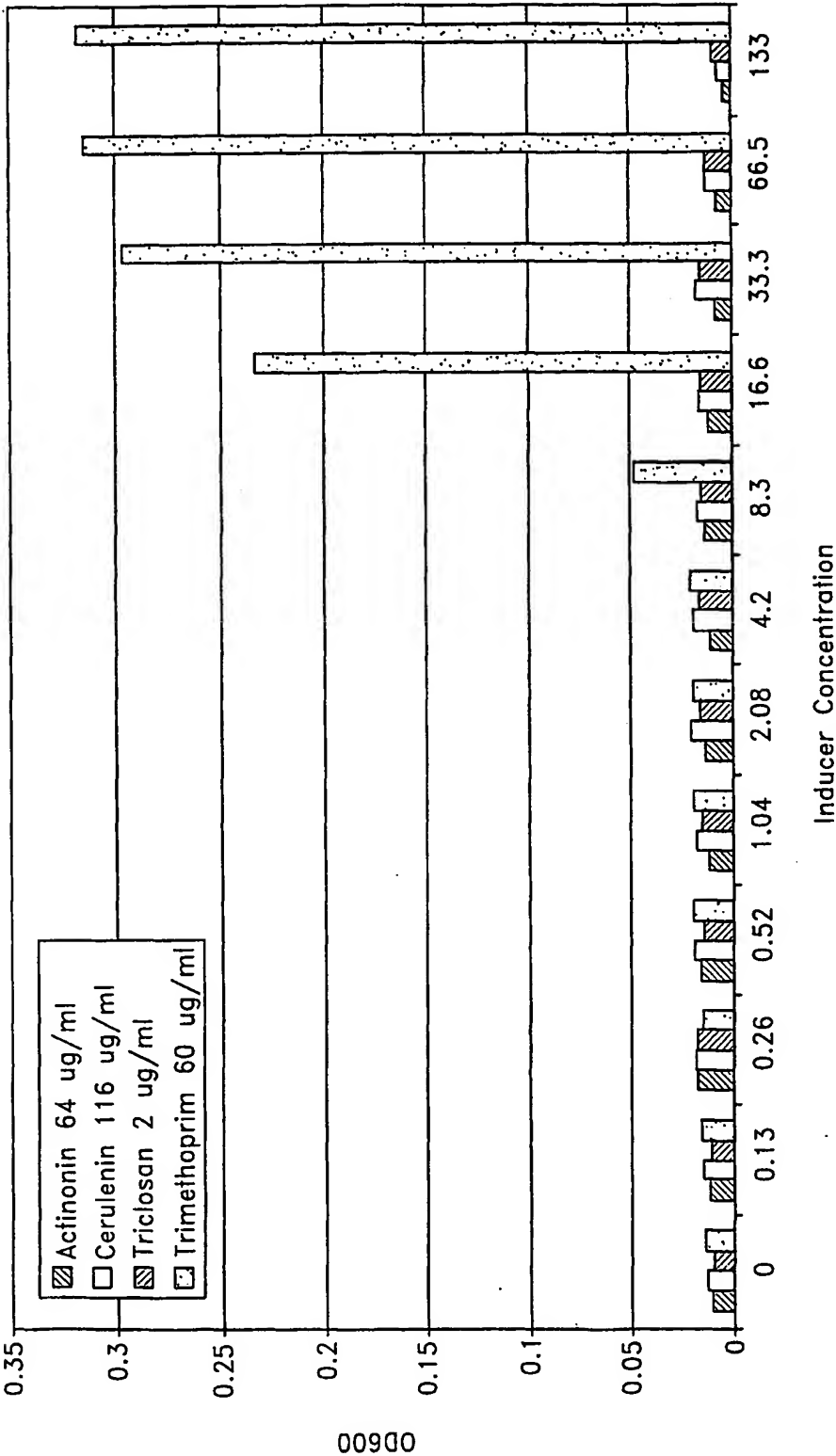


FIG. 15





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FIG. 16

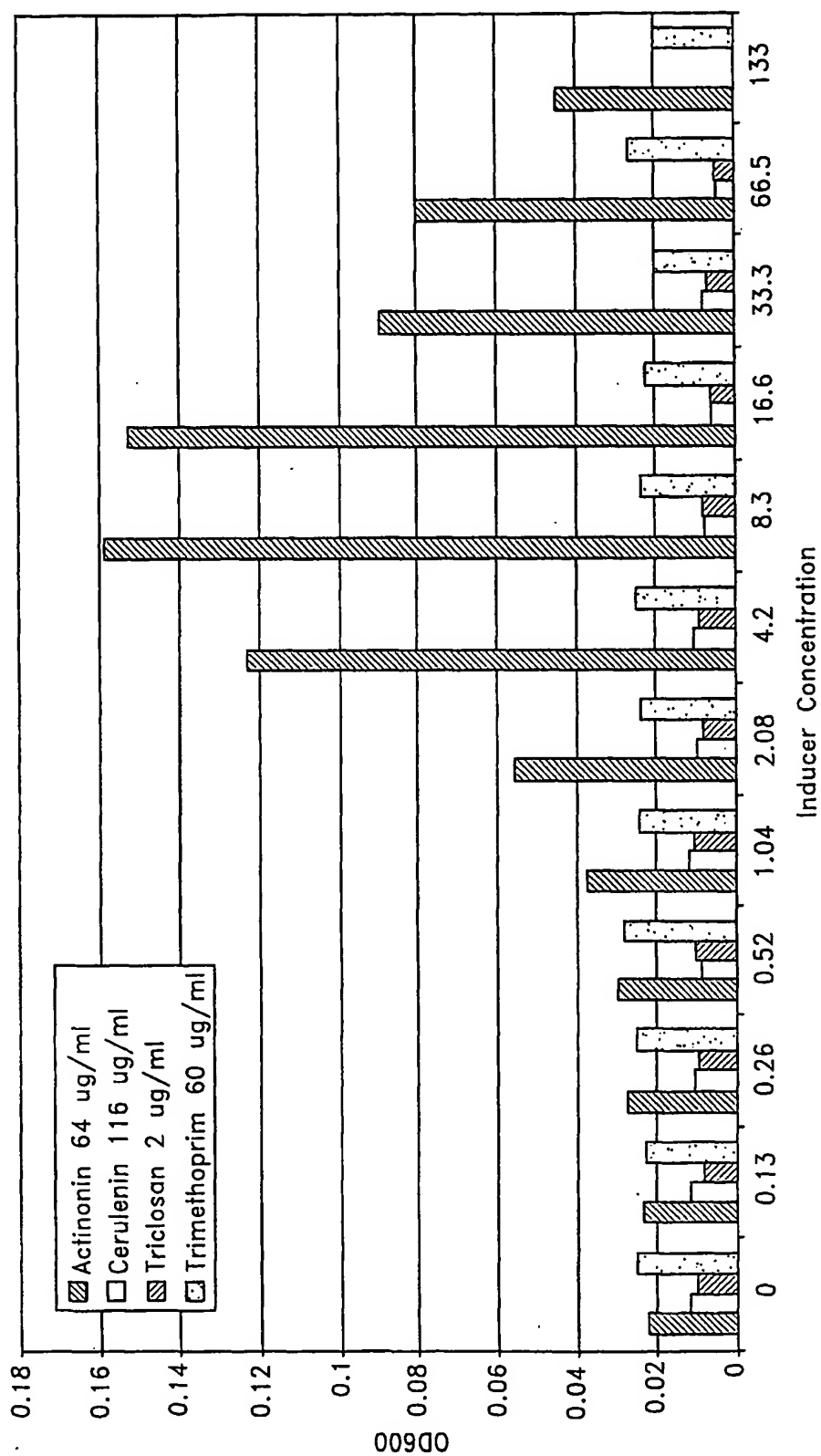


FIG. 17

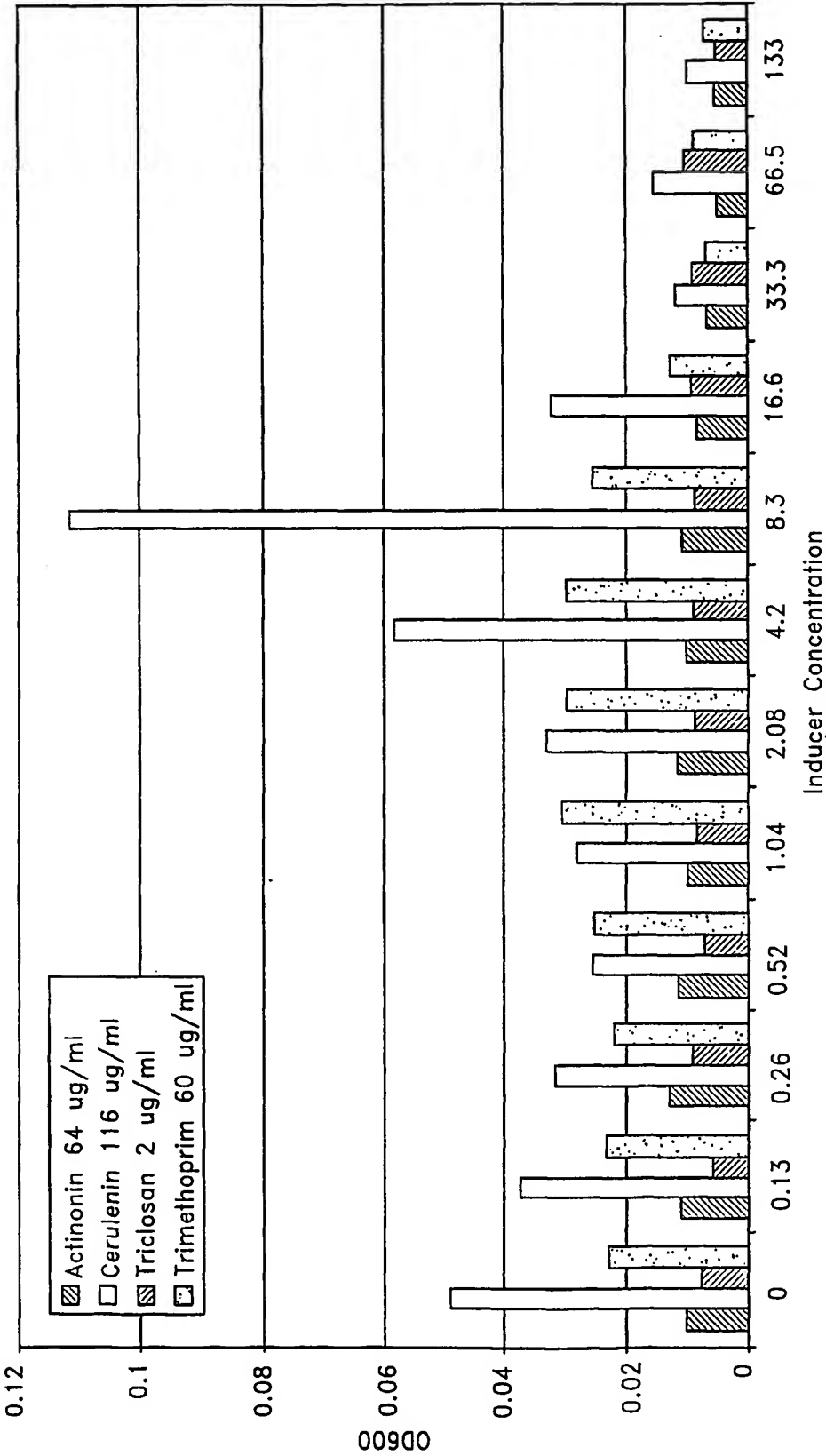


FIG. 18

FIG. 18A

FIG. 18A
FIG. 18B

# Target Clone Amplification in a mixed Culture (Nine Staph Clones)

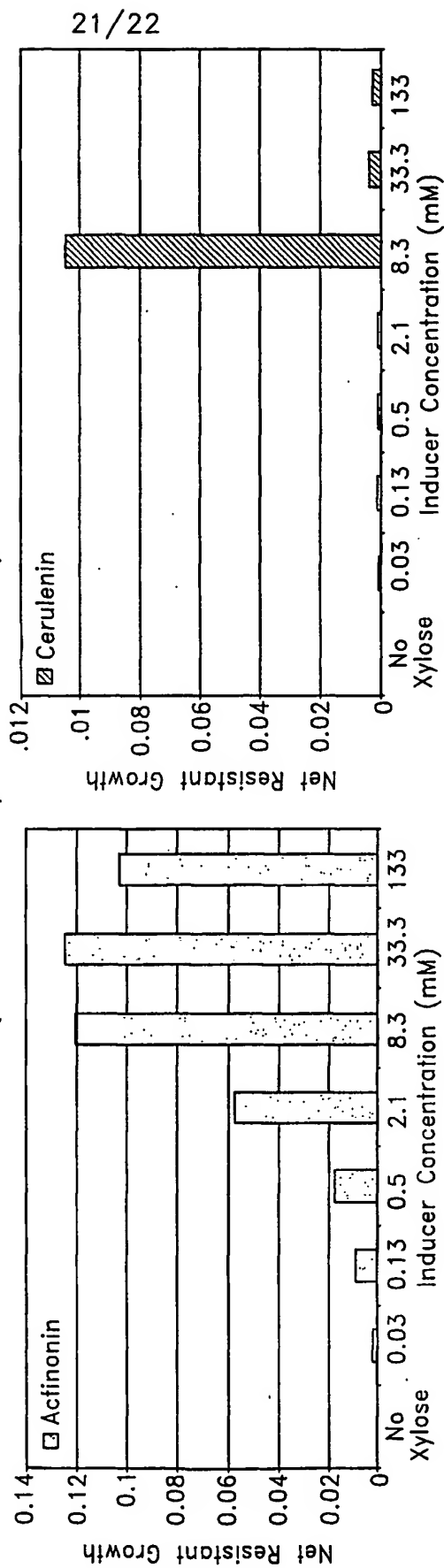
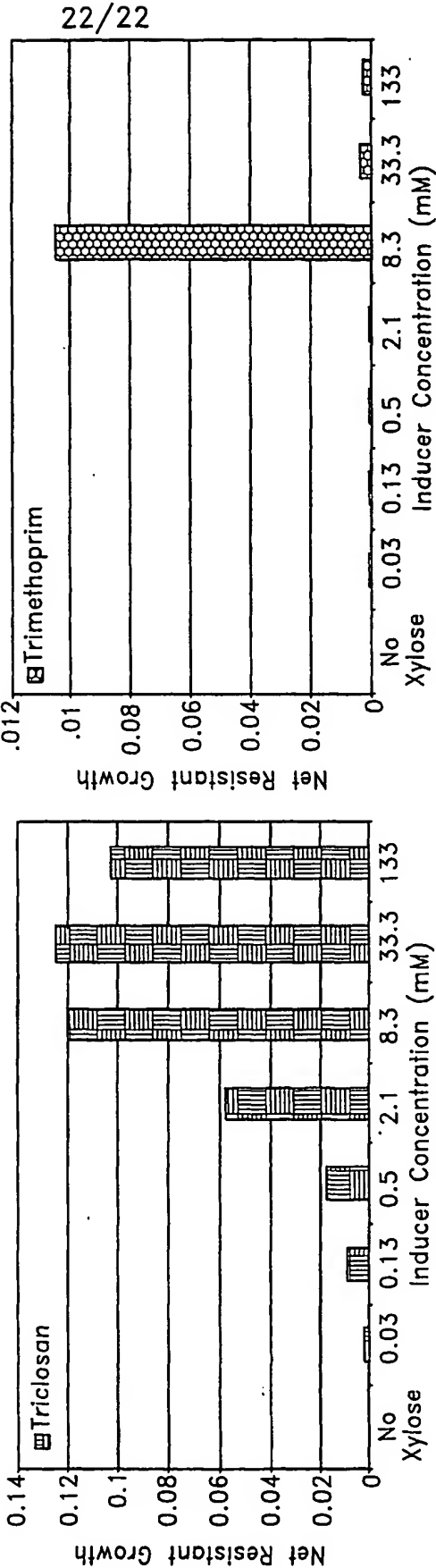


FIG. 18

FIG. 18A
FIG. 18B

FIG. 18B



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